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STUDIES ON BLOOD VISCOSITY AT LOW SHEAR RATES

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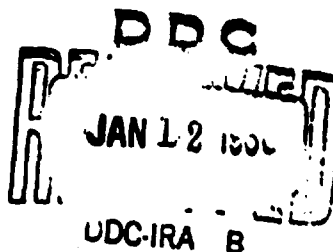
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ABSTRACT

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Blood viscosity was measured at low shear rates in a modified G. D. M. viscometer. At a shear rate of 0.01 sec^{-1} , the time required to reach the maximum torque reading was dependent on hematocrit and plasma protein concentrations. When hematocrit and other plasma proteins were held constant, the rise time was inversely related to the fibrinogen concentration. The shear-rate dependence of blood viscosity was attributed to two factors, namely the interactions between cells and plasma protein which predominated at low hematocrits and the direct cell-cell interactions which became more important at high hematocrits. The addition of albumin to cell suspensions caused a proportionate increase in viscosity at all shear rates, whereas the addition of fibrinogen raised the viscosity preferentially at low shear rates. Suspensions of hardened erythrocytes showed much higher viscosity than normal cell suspensions at the same concentration. Furthermore, filters with 5μ pores allowed the passage of normal cells but not the hardened cells.

The early hemodynamic changes in endotoxin shock in dogs consisted primarily of a marked constriction of small hepatic veins and venules, causing reductions of venous return, cardiac output and arterial pressure. The hepatic venoconstriction was not dependent on sympathetic activity. Following a partial recovery, the cardiac output and arterial pressure decreased again at 40 minutes post-endotoxin. These late changes were more pronounced in dogs with intact spleen which released erythrocytes to raise blood viscosity. The plasma volume decreased progressively in the dogs with spleen but not in the splenectomized dogs.

Pericardial tamponade in splenectomized dogs caused elevation of central venous pressure and decreases of cardiac output and arterial pressure. These changes were maintained when the pericardial pressure was kept high but showed partial recovery when the pericardial pressure was allowed to decrease without supplementary introduction of fluid into the pericardial cavity. The blood volume remained essentially unchanged during tamponade.

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I. INFLUENCE OF COMPONENTS OF BLOOD ON ITS VISCOELASTIC PROPERTIES

In the past year, we have continued and extended our previous investigations (1964 Progress Report) on the influence of blood components on the viscoelastic properties of blood. The information obtained from these studies not only serves to elucidate the physiological determinants of the flow properties of blood but also establishes a baseline for investigations on pathological changes in shock. All viscometric measurements were made in the modified version of a couette viscometer (Gilonson, Dauwalter and Merrill, Trans. Soc. Rheol. 7:319, 1963), as described in the 1964 Progress Report. The shear rate used ranged from 50 to 0.01 sec^{-1} and the temperature was 37°C . Blood samples were obtained from healthy human subjects and mongrel dogs. When whole blood was used for viscometric measurement, heparin was added as the anticoagulant. Ringer suspensions of cells were prepared by washing (3 times) the cells in the heparinized blood samples with Ringer's solution after removing the plasma. Defibrinated blood was made by washing and then suspending Ringer-washed cells in serum, which was obtained after the clotting of non-heparinized blood. In each type of preparation, samples with different cell percentages were prepared by the removal or addition of the respective suspending media.

A. Time Dependence of Viscometric Behavior of Human Blood.

In some preliminary experiments described in the 1964 Progress Report, it was found that when blood viscosity was determined at low shear rates, the torque reading (proportional to shear stress) showed characteristic time dependence. Thus after a blood sample had been thoroughly mixed and introduced into the viscometer, the torque reading obtained with a constant, low shear rate first rose to a maximum and then declined. This time dependence of torque response at low shear rates was a major subject of investigation in the early part of 1965. Fig. 1 shows the alterations in torque reading with time for whole blood, defibrinated blood and Ringer suspensions of cells (all from the same subject with the hematocrit value adjusted to 45% cells) at a constant rate of shear (0.01 sec^{-1}) for 30 minutes. The torque reading for whole blood and defibrinated blood showed an initial rise to a maximum and later decreased. In this sample, low plateau readings were obtained between 20 and 30 minutes, but in some other experiments the torque reading for whole blood showed a secondary, small rise (e.g. Fig. 2). As pointed out in the 1964 Progress Report, the initial rise time was longer for the defibrinated blood (2.5 minutes in Fig. 1) than for the whole blood (1 minute in Fig. 1). The Ringer suspension gave a maximum reading in less than 0.05 minute. The following experiments were performed to evaluate systematically the influence of shear rate, hematocrit and plasma proteins on the torque-time relationship, especially with respect to the initial rise time.

1. Shear Rate and Torque-Time Curve.

Fig. 2 shows the torque-time curves at shear rates ranging from 50 to 0.01 sec^{-1} for a sample of human whole blood (45% hematocrit). At shear rates of 50 and 5 sec^{-1} , there was little time dependence of torque readings. As the shear rate was reduced to 0.5 sec^{-1} and lower, the time dependence became progressively more prominent. Thus the initial rise time became longer and the slope of the subsequent decline of torque became steeper.

Results obtained on the defibrinated blood (45% hematocrit) were qualitatively similar but quantitatively different. Thus when the shear rate was reduced to below 0.05 sec^{-1} , the initial rise time for the defibrinated blood was much more prolonged than that for the whole blood. In Fig. 3, the relation of the initial rise time to shear rate is shown for the whole blood, defibrinated blood and Ringer cell suspensions. It can be seen that with shear rates above 0.2 sec^{-1} , the initial rise time was only of the order of 0.1 minute, regardless of the shear rate and the presence of plasma proteins. Since similar order of response time was observed for the standard oils obtained from the National Bureau of Standards, this short time interval represented the delay inherent in the instrument. At shear rates lower than 0.1 sec^{-1} , the initial rise time for Ringer suspensions rose slightly, but was not significantly different from that for the standard oils. At the lowest shear rates, the prolongation of the initial rise time was more significant for the whole blood and most prominent for the defibrinated blood (Fig. 3). Thus at a shear rate of 0.01 sec^{-1} , the initial rise time averaged 0.3 minute for Ringer suspensions of cells, 0.9 minute for whole blood and 2.6 minutes for defibrinated blood.

2. Hematocrit and Torque-Time Curve.

At any given low shear rate, the time dependence of torque response became increasingly pronounced as the hematocrit was raised. For whole blood, defibrinated blood and Ringer suspensions of cells the initial rise time at 45% hematocrit and 0.05 sec^{-1} was under 0.5 minute (Fig. 4 lower panel); but when the hematocrit was elevated to 98% the initial rise time was prolonged to approximately 1 minute for all three types of samples. The influence of hematocrit value (upper panel of Fig. 4) on the initial rise time was much stronger at 0.01 sec^{-1} . For defibrinated blood, the prolongation of the rise time was most prominent when the hematocrit was raised from 25% to 45%. For whole blood, the effect was most marked between hematocrit values of 45% and 70%. For Ringer suspensions, major change occurred only when the hematocrit was raised above 70%. Although the rise-time vs. hematocrit relationship at 0.01 sec^{-1} was different for the three types of preparations, the rise time at a hematocrit of 98% was essentially the same, regardless of the composition of the suspending medium.

3. Fibrinogen and Torque-Time Curve.

From the results described above, it is seen that defibrination caused a major alteration in the torque-time relationship, especially at a shear rate of 0.01 sec^{-1} and a hematocrit of 45% (Figs. 3 and 4). The following experiments were conducted to investigate (a) whether the effect of defibrination was due to the absence of fibrinogen or due to the removal of other clotting elements during defibrination, and (b) the quantitative relationship between fibrinogen concentration and the initial rise time.

Fibrinogen was isolated and purified from human plasma according to the method used by Dr. E.B. Reeve and his co-workers at the University of Colorado Medical Center (Reeve and Roberts, J. Gen. Physiol. 43:414, 1953; Takeda, Am. J. Physiol. 206:1223, 1964). The purified fibrinogen was dissolved in autologous heparinized serum to yield fibrinogen concentrations varying from 0 to 1.2 g%. Autologous erythrocytes were washed with Ringer's solution twice and then once with the fibrinogen-serum medium before finally resuspended in the latter to yield a hematocrit of 45%. The fibrinogen concentration in the suspending media of the final samples was determined by the method of Ratnoff and Menzie (J. Lab. Clin. Med. 37: 313, 1951). In Fig. 5, the logarithm of the initial rise time is plotted against the fibrinogen concentration. As the fibrinogen concentration was increased from 0 to 0.5-0.6 g%, the rise time became progressively shortened and a linear relationship existed on the semi-log plot. The initial rise time was reduced to 0.2-0.3 minutes with fibrinogen concentrations of 0.5-0.6 g% and did not decrease significantly with further elevation of fibrinogen concentration to 1.2 g%.

The fibrinogen concentration in the original plasma samples averaged 0.28 g%. Reference to Fig. 5 shows that the rise time in the reconstituted blood samples containing 0.28 g% of fibrinogen in the suspending medium is approximately 0.9 minutes. This is the same time interval obtained in the original whole blood without defibrination (Fig. 3). Therefore the addition of purified autologous fibrinogen to defibrinated blood can reduce the initial rise time to a value comparable with that obtained in whole blood. It is concluded that the difference in the initial rise time between whole blood and defibrinated blood can be explained on the basis of the presence or absence of fibrinogen.

4. Summary and Comments.

When whole blood was subjected to a constant shear rate lower than 0.5 sec^{-1} , the shear stress recorded showed a time dependence. The time required to attain a maximum shear stress became progressively prolonged at lower shear rates and higher hematocrits. The removal of all plasma proteins reduced the initial rise time but the removal of only fibrinogen prolonged the rise time. At a shear rate of 0.01 sec^{-1} and a hematocrit of 45%, the rise time was inversely related to the fibrinogen concentration. Because of the time dependence of the torque response at low shear rates, the viscometric behavior of blood actually cannot be described by a single viscosity value. The viscosity value in the following sections of this Report refers to that calculated from the maximum torque value. Since the rise time shows marked dependence on plasma proteins and especially fibrinogen, it may be an important rheological parameter to consider in shock when the concentrations of fibrinogen and other plasma proteins are altered.

B. Influence of Plasma Proteins on Blood Viscosity.

1. Contribution of Cell-Protein Interactions to the Non-Newtonian Behavior of Human Blood.

In the 1964 Progress Report, the viscosity of human whole blood was compared with Ringer suspensions of cells at shear rates from 52 to 0.052 sec^{-1} . These investigations have been extended to include defibrinated blood. As shown in Fig. 3A, at any given shear rate and hematocrit, the defibrinated blood (solid lines) showed lower viscosity than the whole blood (dotted lines), indicating that the presence of fibrinogen caused an increase of blood viscosity. Fig. 3B shows the comparison between whole blood (dotted lines) and Ringer suspensions of cells (solid lines). The removal of all plasma proteins caused a further decrease of viscosity than the removal of fibrinogen alone. In Fig. 7, the relationship between shear rate and viscosity is shown as log-log plots for the various systems at hematocrit values of 0, 45 and 90%. Plasma, serum, and Ringer solution ($H = 0$) were Newtonian, i.e. their viscosities were independent of shear rates. At 45% hematocrit, dependence of viscosity on shear rate was almost absent in Ringer suspensions of cells, moderate in defibrinated blood, and most pronounced in whole blood. At 90% hematocrit, the viscosities of all three systems were strongly and almost equally shear-rate dependent. These results (Figs. 3 and 7) indicate that at low hematocrit values the non-Newtonian behavior of blood is due primarily to the presence of plasma proteins, partially fibrinogen and partially the other proteins. At high hematocrit values (e.g. 90%), the non-Newtonian behavior is attributable primarily to direct interactions among cells, without the necessity of the presence of plasma proteins.

Changes in blood viscosity and shear stress as the shear rate approaches zero are important for ascertaining whether or not blood can sustain a yield stress before flow begins. As depicted in Fig. 7, down to a shear rate of 0.052 sec^{-1} , the viscosity of whole blood at a normal hematocrit apparently showed a continually upward trend, suggesting the existence of a yield point. However, this suggestion was not supported by measurements made at still lower shear rates. When measurements were extended down to a shear rate of 0.01 sec^{-1} , the log viscosity-log shear rate plot gave a sigmoidal curve tending to reach plateaus at both ends of the shear rates studied (Fig. 3). Other investigators have found that, at sufficiently high shear rates (above $200\text{--}400 \text{ sec}^{-1}$), blood is similar to a Newtonian fluid with a constant, low viscosity (Cerny *et al.*, *Am. J. Physiol.* 202:1188, 1962). The present experiments on shear rates down to 0.01 sec^{-1} suggest that at very low rates blood again approaches another Newtonian region with high viscosity. If such a second Newtonian region with a constant viscosity exists, then at zero shear rate the shear stress (viscosity \times shear rate) also would be zero without a yield stress. In order to study the changes in shear stress as the shear rate approaches zero, rheological data on blood often have been presented in the form of the square root of shear stress ($\tau^{1/2}$) versus the square root of shear rate ($\dot{\gamma}^{1/2}$) (Casson, in *Rheology of Dispersed Systems*, ed. by C.C. Mills, 1959, Pergamon; Cokelet *et al.*, *Trans. Soc. Rheol.* 7:303, 1963), a plot in which the low ends of the coordinates are mathematically expanded for detailed examination. If a linear relationship exists between these two variables, then

$$\tau^{1/2} = \tau_y^{1/2} + b^{1/2} \cdot \dot{\gamma}^{1/2} \quad (1)$$

In equation 1, $\tau_y^{1/2}$ is the intercept on the $\dot{\gamma}^{1/2}$ axis at zero shear rate, and τ_y thus represents the yield stress of the system. As shown in Fig. 9, for whole blood $\tau^{1/2}$ was not a simple linear function of $\dot{\gamma}^{1/2}$. Instead the $\tau^{1/2}$ - $\dot{\gamma}^{1/2}$ curve, down to a shear rate of 0.01 sec^{-1} , showed an everchanging slope convexing to the $\tau^{1/2}$ axis. In such a plot, any reasonable extrapolation would pass through the origin (Fig. 9). Therefore if measurements are made at sufficiently low shear rates, whole blood does not have a yield stress under the conditions of our experiments. In the absence of such low shear rate measurements, one may get the impression that the terminal portion of the $\tau^{1/2}$ - $\dot{\gamma}^{1/2}$ plot is linear. If a linear extrapolation is made for such a short portion of the curve, an "apparent yield stress" would be obtained. In spite of our findings that blood had no yield stress, the $\tau^{1/2}$ and $\dot{\gamma}^{1/2}$ data at two shear rates (0.52 and 0.052 sec^{-1}) were fitted to equation 1. Extrapolation of a straight line connecting these two points to zero shear rate gave an intercept of $\tau_e^{1/2}$. This was done for two reasons: (a) to compare our results with those of other investigators (Cokelet *et al.*, Trans. Soc. Rheol. 7:307, 1963; Merrill *et al.*, Biophys. J. 3:199, 1963) who have used equation 1 to analyze their rheological data, and (b) to gain an empirical, though arbitrary, measure of the degree of departure from Newtonian behavior for the various systems. The τ_e values thus calculated can be correlated with hematocrit. For whole blood and defibrinated blood, with hematocrits up to 80% (Fig. 10A), the cube root of τ_e had a linear relationship with the hematocrit. These results are similar to those obtained by Merrill *et al.* (Biophys. J. 3:199, 1963). At high hematocrit values, the relationship between τ_e and hematocrit was better described by an exponential function given in Fig. 10B. It is seen from Fig. 10A that the hematocrit

values at which \mathcal{T}_g became appreciable were approximately 3% for whole blood, 20% for defibrinated blood, and 30-40% for Ringer suspensions of cells. These values were approximately the hematocrits at which non-Newtonian behavior first appeared with increasing hematocrit (Figs. 6A and 6B). It is also indicated in Fig. 10A that, up to the normal hematocrit range, the \mathcal{T}_e in whole blood was primarily due to the presence of plasma proteins (especially fibrinogen) which interacted with the cells. When the hematocrit was progressively increased, the \mathcal{T}_e values for the three systems (whole blood, defibrinated blood, and Ringer suspensions of cells) became close to one another, indicating the predominance of cell-cell interactions over cell-protein interactions. Therefore the relative contributions by these two types of interactions (cell-protein and cell-cell) to the empirical values \mathcal{T}_e were similar to their relative contributions to the non-Newtonian behavior of whole blood, and the value \mathcal{T}_e can be used as a semi-quantitative description of the non-Newtonian behavior of blood systems.

2. Effects of Albumin and Fibrinogen on the Viscosity of Ringer Suspensions of Dog Cells.

In October and November of 1965 a series of collaborative experiments were performed with Dr. Luddo Nanninga (from the laboratory of Dr. Mason Guest at the University of Texas College of Medicine). These experiments were designed to investigate the influence of albumin and fibrinogen on the viscosity of dog cell suspensions. A comparison was also made between two types of bovine fibrinogens, one with profibrinolysin and the other without profibrinolysin.

a. Effects of Albumin. Purified dog albumin (Pentax Co.) was added to Ringer suspensions of dog cells. The final albumin concentrations in the suspensions ranged from 2 to 3 g% and the hematocrit was 45%. At any given shear rate the addition of albumin increased the viscosity value, and the increase can be correlated with the albumin concentration in the suspension (Fig. 11). When the viscosity-shear rate relationship was examined, it can be seen that the addition of albumin caused a proportionate increase in viscosity at all shear rates, and there was no significant change in the non-Newtonian behavior of the suspensions (Fig. 12).

b. Effects of Dog Fibrinogen. Dog Fibrinogen was isolated and purified (Takeda, Am. J. Physiol. 206:1223, 1964) and added to the Ringer suspension of autologous dog cells. The final fibrinogen concentration in the suspending medium ranged from 0 to 0.5 g% and the hematocrit was 45%. The addition of dog fibrinogen to the Ringer suspension of cells caused a progressive

increase in viscosity (Fig. 13), especially at low shear rates. At a shear rate of 50 sec^{-1} the viscosity of the suspensions containing various amounts of fibrinogen did not differ appreciably from that of the suspensions containing no fibrinogen. At a shear rate of 0.05 sec^{-1} the presence of 0.23 g% fibrinogen raised the viscosity to 4.5 times control. Still larger rise in viscosity was obtained with further increases of fibrinogen concentration. Therefore fibrinogen alone, in the absence of other plasma proteins, can cause an increase in the viscosity of cell suspension. In contrast to the albumin, which caused a proportionate increase of viscosity at all shear rates, fibrinogen increased viscosity preferentially at the low shear rates. Therefore fibrinogen is an important factor in causing the non-Newtonian behavior of blood.

c. Effects of Bovine Fibrinogen. Two types of bovine fibrinogen preparations were made by Dr. Nanninga's group; one contained profibrinolysin and the other was free of profibrinolysin. These fibrinogen preparations were added to the dog cell suspensions to yield concentrations in the suspending media ranging from 0 to 0.3 g%. The addition of either fibrinogen preparation caused an increase in viscosity of the suspension, especially at the low shear rate range. When equivalent fibrinogen concentrations were added, the two types of bovine fibrinogen preparations showed no significant difference in their effects on the viscosity of cell suspensions (Fig. 14). As in the case of the addition of dog fibrinogen, bovine fibrinogen raised the viscosity primarily

at low shear rates (Figs. 14 and 15). When the effects of bovine fibrinogen on the viscosity of dog cell suspensions (Fig. 15) were compared with those of the autologous dog fibrinogen (Fig. 13), there was no significant difference. Therefore the species difference in fibrinogen as well as the presence or absence of profibrinolysin did not affect the influence of fibrinogen on the viscosity value and the non-Newtonian behavior of dog cell suspensions.

d. The Combined Effects of Albumin and Fibrinogen. As shown in Fig. 12, the effect of adding both albumin and fibrinogen to the dog cell suspensions was approximately equal to the sum of the individual actions of these two proteins when added alone. Therefore the effects of these two proteins are additive.

C. Erythrocyte Deformability.

1. Influence of Deformability on Blood Viscosity--Studies on Hardened Cells.

In the previous year, experiments were performed to compare the viscosity of suspensions of rigid polystyrene latex particles and Ringer suspension of human cells (1934 Progress Report). At any given shear rate and particle concentration the viscosity was considerably higher in the latex suspensions, suggesting that the lack of particle deformability contributed to a higher viscosity. Of course, latex particles differ from human erythrocytes not only in deformability but also in size, shape and other physico-chemical properties. In order to investigate more directly the influence of deformability on viscosity of cell suspension, viscosity was measured on suspensions of dog cells hardened with acetaldehyde (Heard and Seaman, J. Gen. Physiol. 43:335, 1960) and compared with that of suspensions of normal, deformable dog cells. These experiments were done in September 1935 in collaboration with Dr. Geoffrey Seaman of the Department of Radiotherapeutics, University of Cambridge. Washed dog red cells were suspended in acetaldehyde solution for over 3 weeks. During this time cross linkages were formed among hemoglobin molecules, such that the erythrocyte became rigid and no longer deformable. The hardened cells were then washed and suspended in saline solution to give cell concentrations ranging from 0 to close to 30%. The viscosity of these suspensions as well as the suspensions of normal dog cells was determined at shear rates from 50 to 0.05 sec^{-1} . As shown in Fig. 13, the viscosity of suspensions of hardened dog cells did not show marked dependence on the shear rate. When the cell concentration was higher than 30%,

the viscosity of hardened dog cell suspensions at any given shear rate and cell concentration was much higher than that of the normal cell suspensions. These experiments comparing the hardened dog cells with deformable dog cells, both being suspended in the same medium, offers a direct proof that the deformability of red cells plays an important role in lowering blood viscosity and facilitating blood flow. In the suspensions of normal dog cells, viscosity measurements can be made with cell percentages as high as 98%. In contrast, the viscosity of hardened cells containing more than 50% cells rose so sharply that the viscosity at 30% cells was beyond the upper limit of measurement in our instrument.

2. Measurement of Deformability by Microfiltration.

There is abundant visual evidence that normal red cells undergo deformation in the blood stream (e.g. Guest et al., Science 142:1312, 1963; Branemark et al., Biorheology 1:139, 1963; and others), and Frothero and Burton (Biophysical Journal 2: 213, 1962, "The Pressure Required to Deform Erythrocytes in Acid-Citrate-Dextrose") have reported that red cells readily pass through filters of 5 and even 3 μ pore size under low pressures. Last spring one of us (M.I.G.) made numerous unsuccessful attempts to confirm the experiments reported by Frothero and Burton. When we consulted with Dr. Burton we learned that he too had been unable to repeat their earlier results.

In recent months we have tested metallic silver filters 50 μ thick (Celas Flo-tronics) and polycarbonate sieves 10 μ thick (Fleischer et al., Science 149:383, 1965) both with nominal pore size of 5 μ . Flow rates of the Ringer's solution used as diluent were determined for each filter at a specified head of pressure. The pressure was controlled by (a) maintaining a fluid column of known height above the filter (positive pressure) or (b) maintaining a constant pressure below atmospheric beneath the filter (negative pressure). Next, cell suspensions were passed through the filter and flow rate obtained as a function of volume filtered. The erythrocyte concentration in the original suspension and the filtrate was determined in a Coulter Electronic Counter. Figures 17 and 18 show the results of typical experiments, where flow rate and erythrocyte concentration have been plotted against volume passed. Normal dog or human erythrocytes suspended in Ringer's solution at concentrations ranging from 1000 to 100,000 cells/cu mm come through these sieves at nearly 100% of the original

at filtration pressures of 10-11 cm H₂O (see Figs. 17 and 18). With hardened red cells (Heard and Seaman, Biochim. Biophys. Acta 53:360, 1961) the filtrate contained no cells detectable with a Coulter Counter, which provides evidence that the pores are smaller than the red cells.

With suspensions of normal blood cells, the straight line on the semilogarithmic plot indicates that the data fit closely an equation of the form

$$R = R_0 \exp (-\lambda v),$$

where R_0 is the initial flow rate and v is the volume passed. The parameter λ , which is the slope of the line, is a measure of the "clogging rate" of pores in the filter.

It is likely that the value of λ will depend on the cell concentration, size and distribution of pores in the filter, and on the pressure difference across the filter. Other factors, such as cell size, rigidity, electric charge, etc. may also influence λ . Hence, this experimental approach seems promising as a tool for the study of factors influencing the flow properties of blood.

Thanks to the cooperation of several industrial laboratories, we hope soon to have additional filters and/or sieves of greater uniformity and with certain characteristics that our tests so far indicate may be useful.

Dr. Cyrus Bryant assisted in these filtration experiments.

Fig. 1

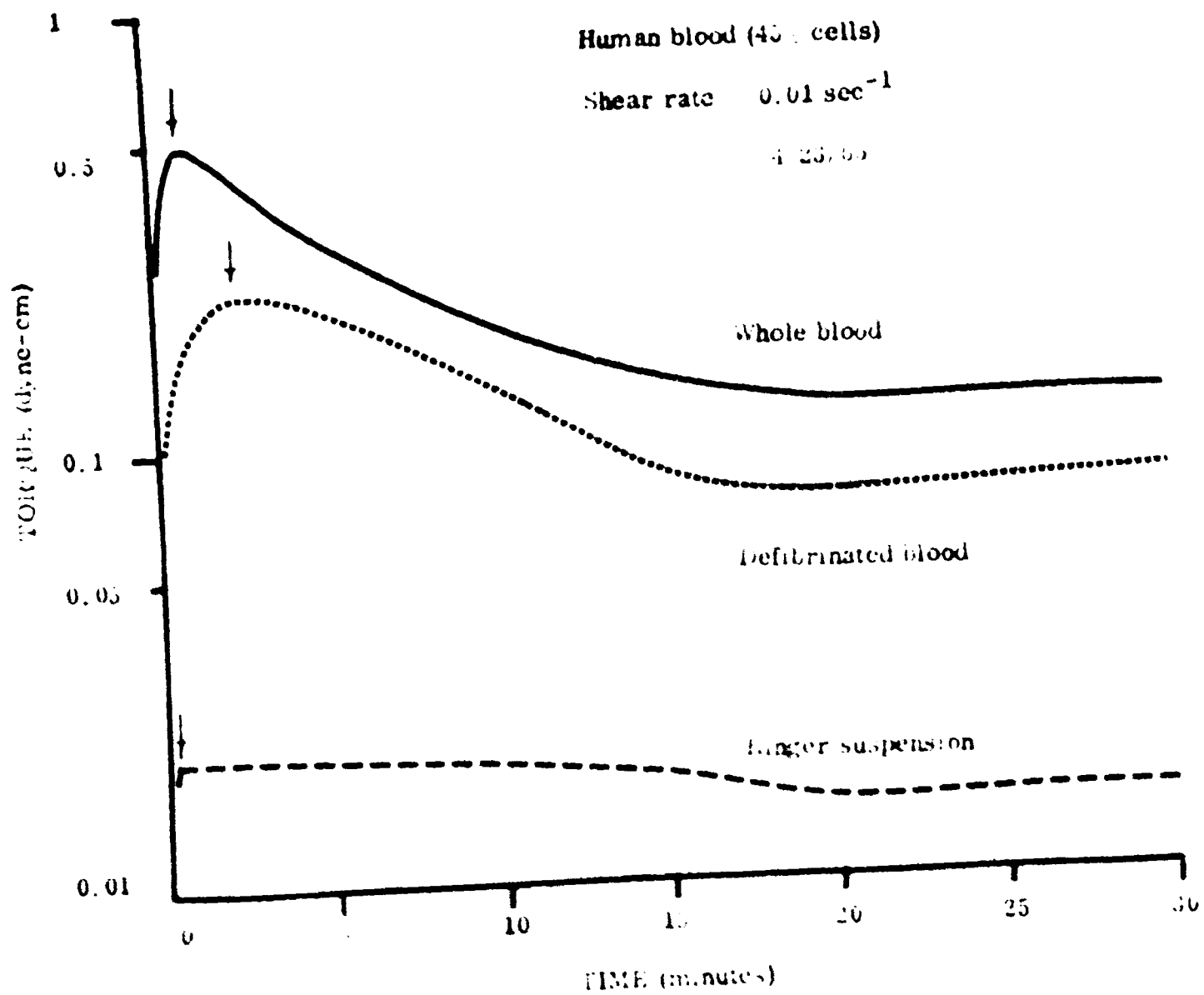


Fig. 2 Human whole blood (3/11/65)
45% cells

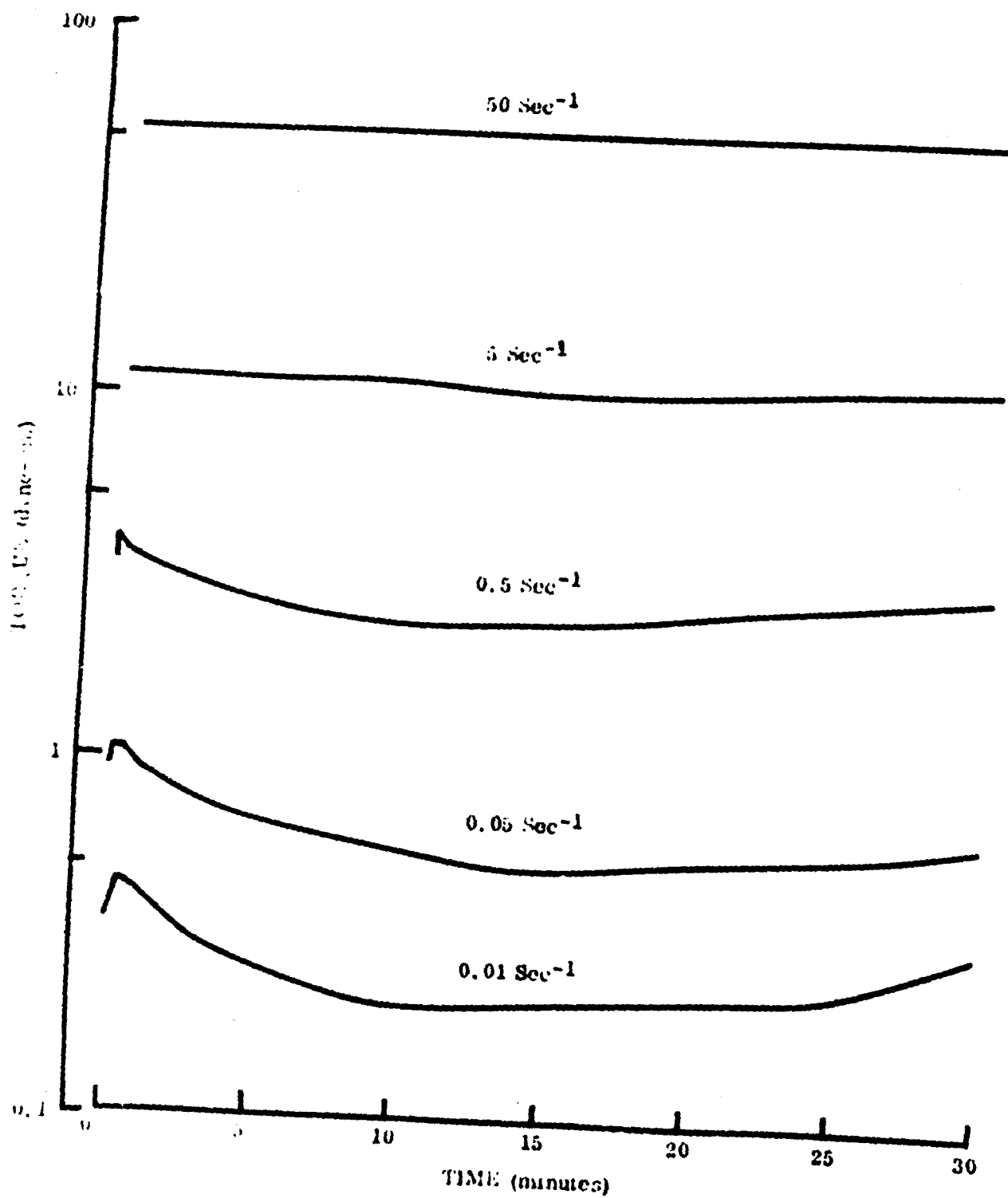
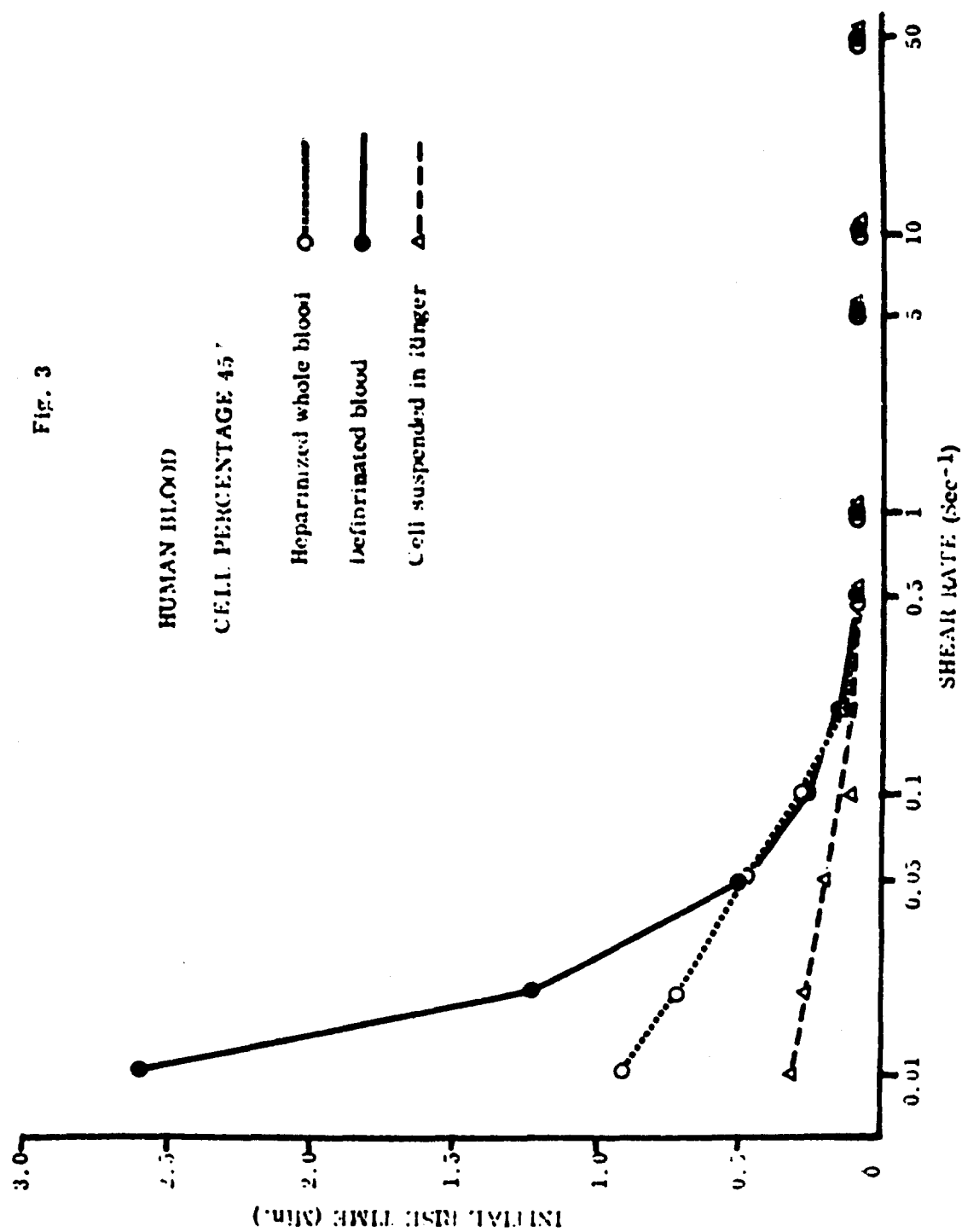


Fig. 3



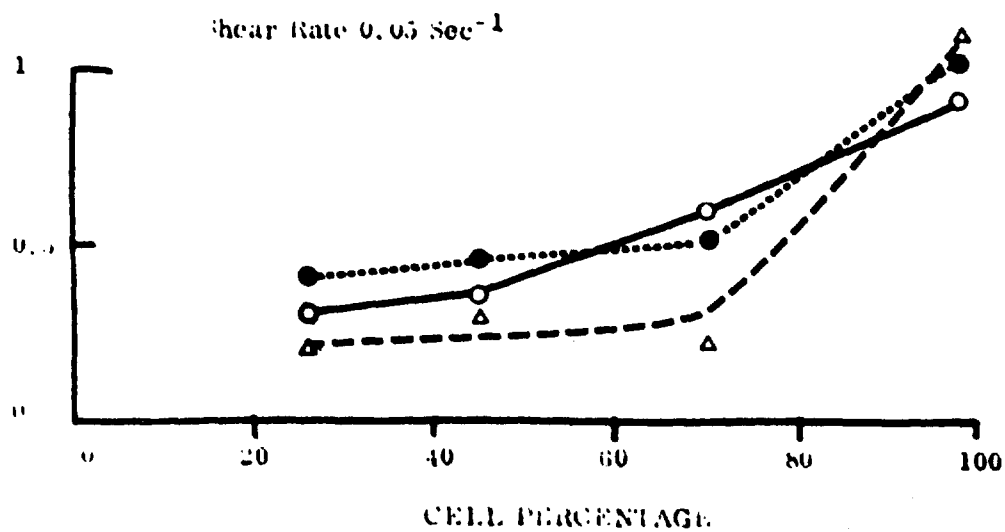
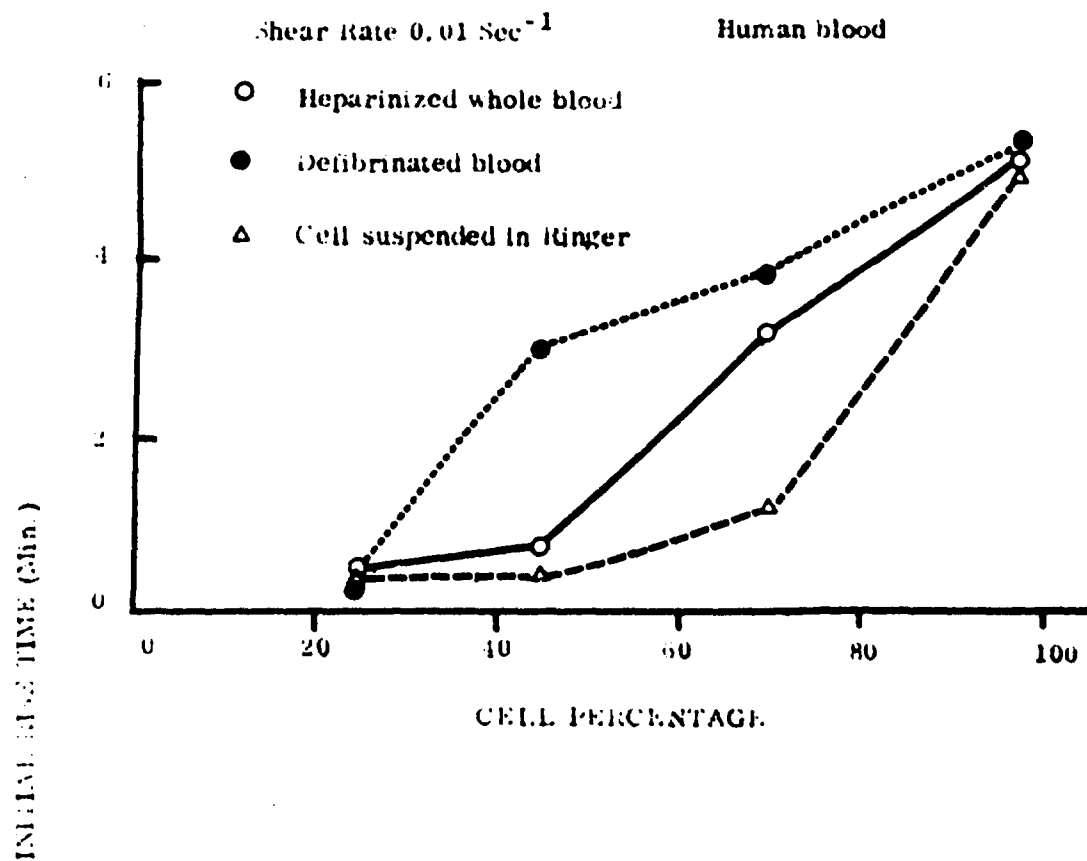
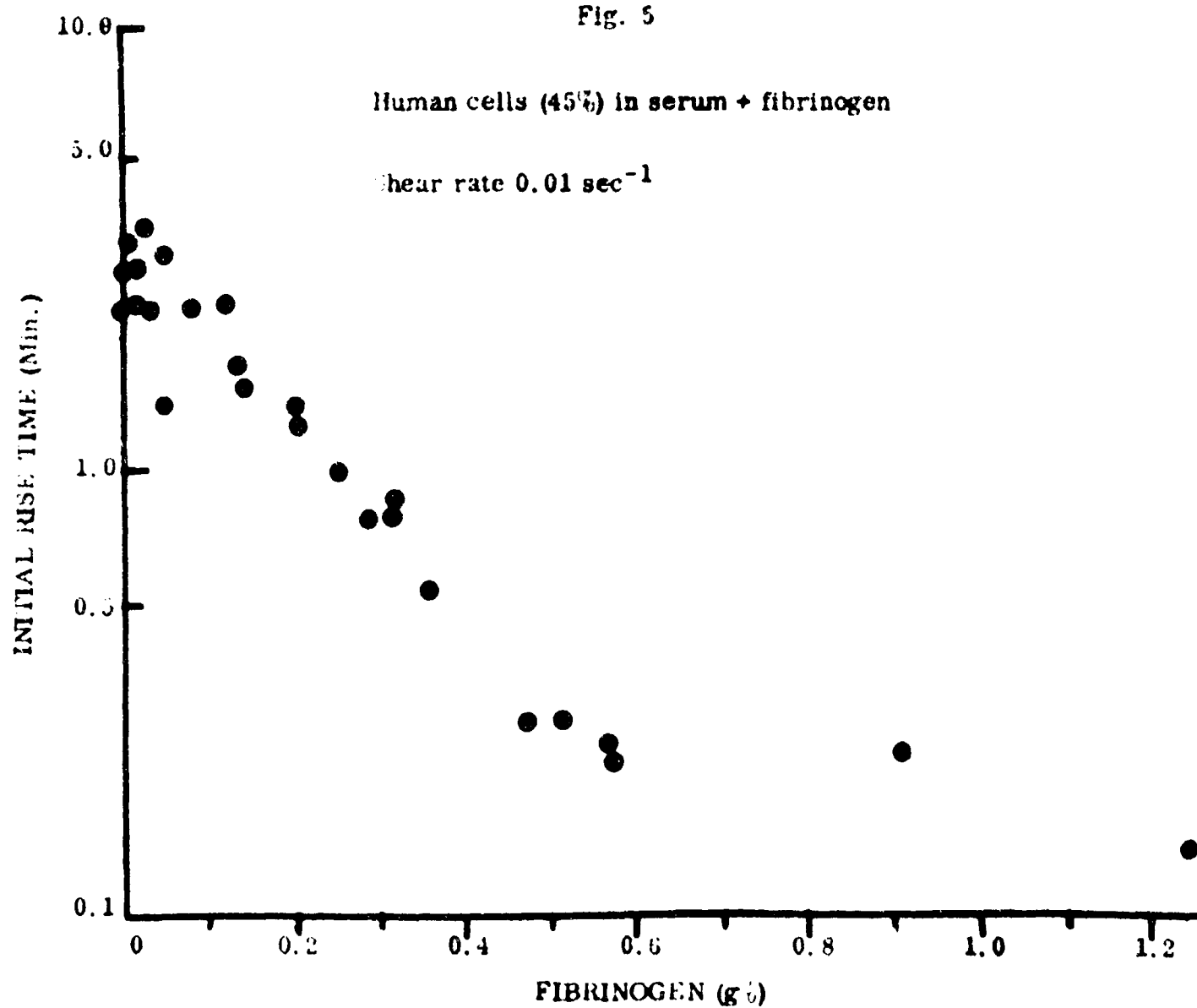


Fig. 4

Fig. 5



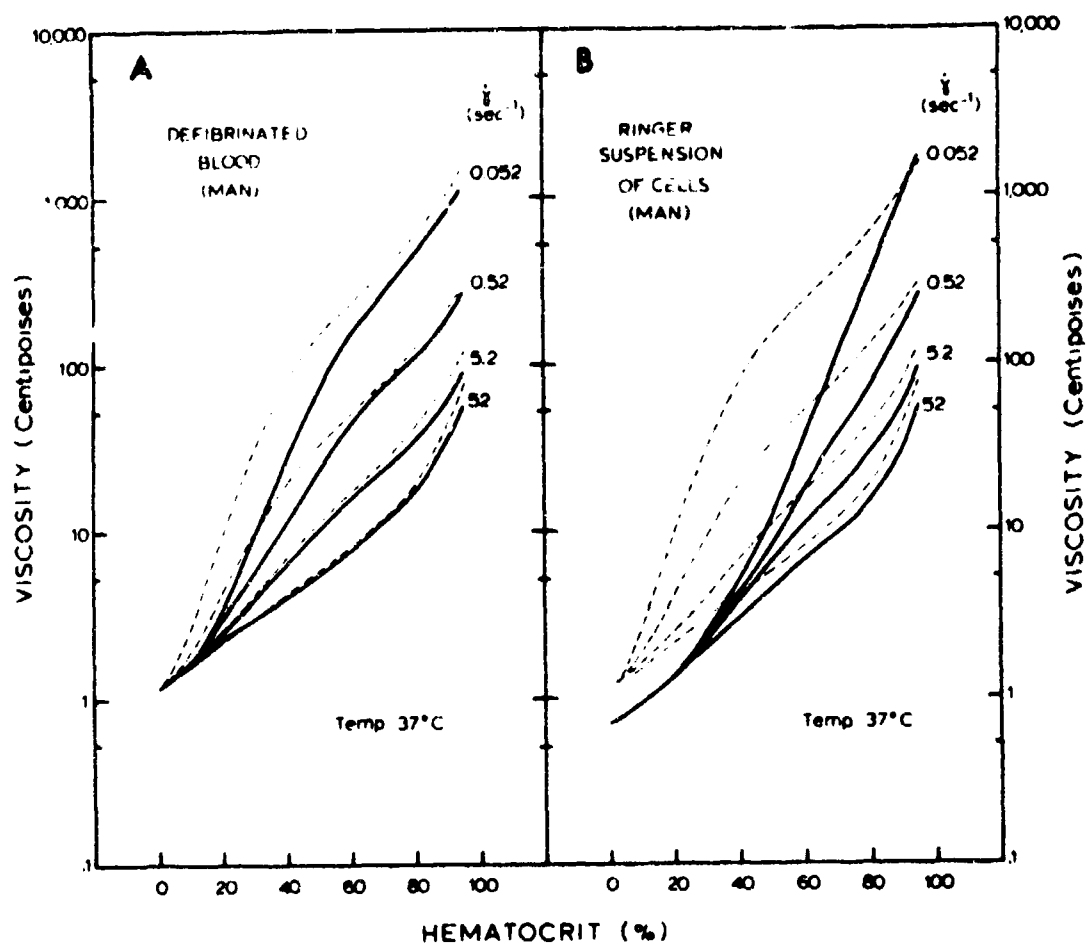


Fig. 6. Relationship between the logarithm of viscosity at four shear rates and the hematocrit in defibrinated blood (A, solid lines) and Ringer suspensions of cells (B, solid lines). The dotted lines in A and B represent the results on whole blood.

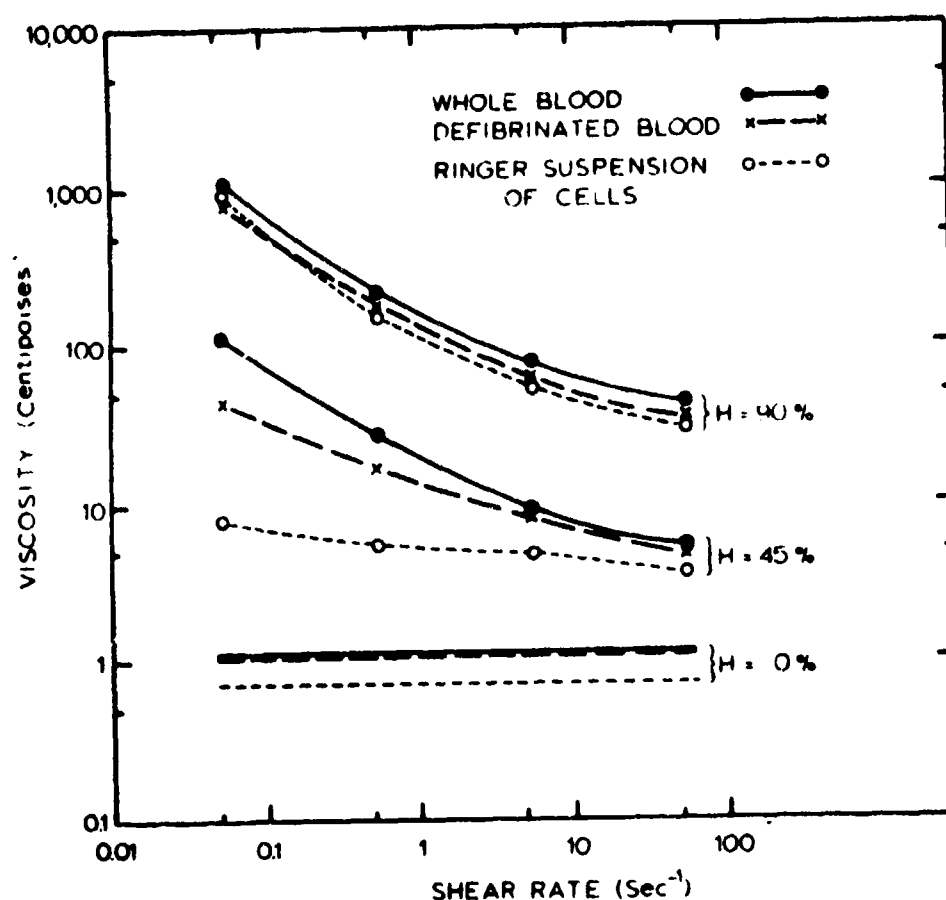


Fig. 7. Relationship between the logarithm of viscosity and the logarithm of shear rate for whole blood, defibrinated blood and Ringer suspensions of cells at hematocrits (H) of 90 and 45%. The lines marked H = 0 represent the data on plasma (solid line), serum (broken line) and Ringer's solution (dotted line). Note the Newtonian behavior of the suspending media and the relative extents of departure from Newtonian behavior in the three blood systems at H = 45% and at H = 90%.

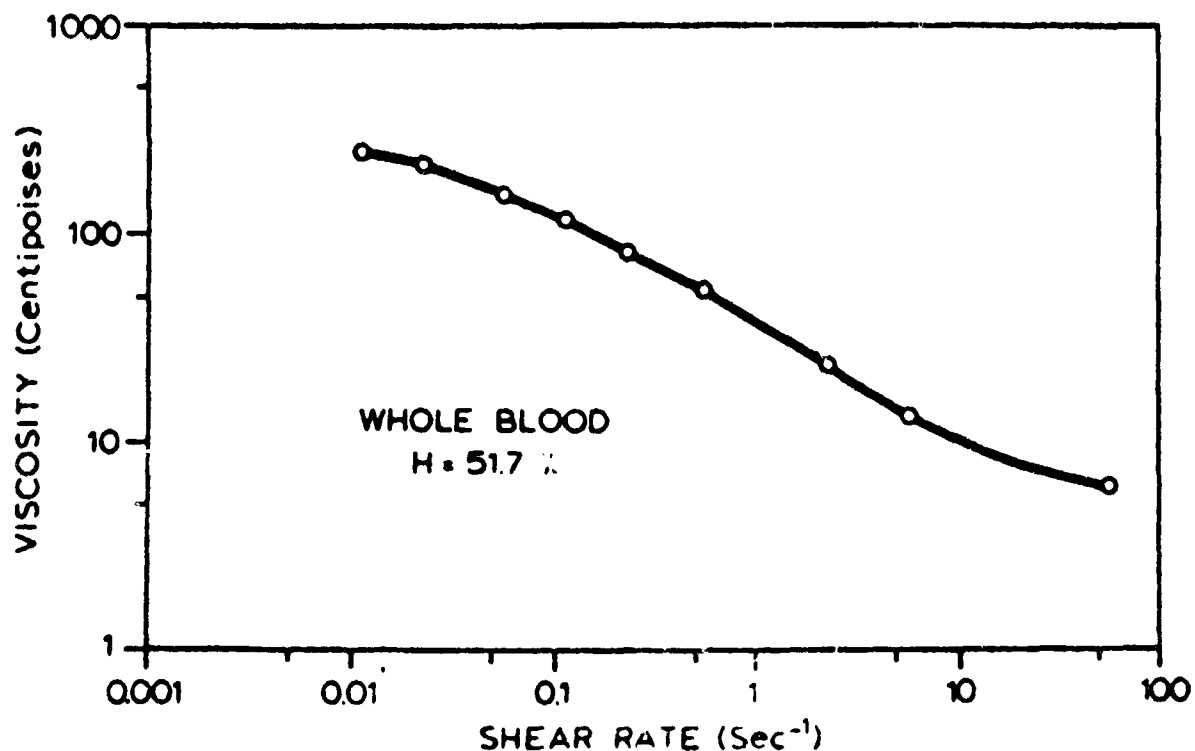


Fig. 8. Relation between the logarithm of viscosity and the logarithm of shear rate (down to 0.01 sec⁻¹) in a sample of whole blood.

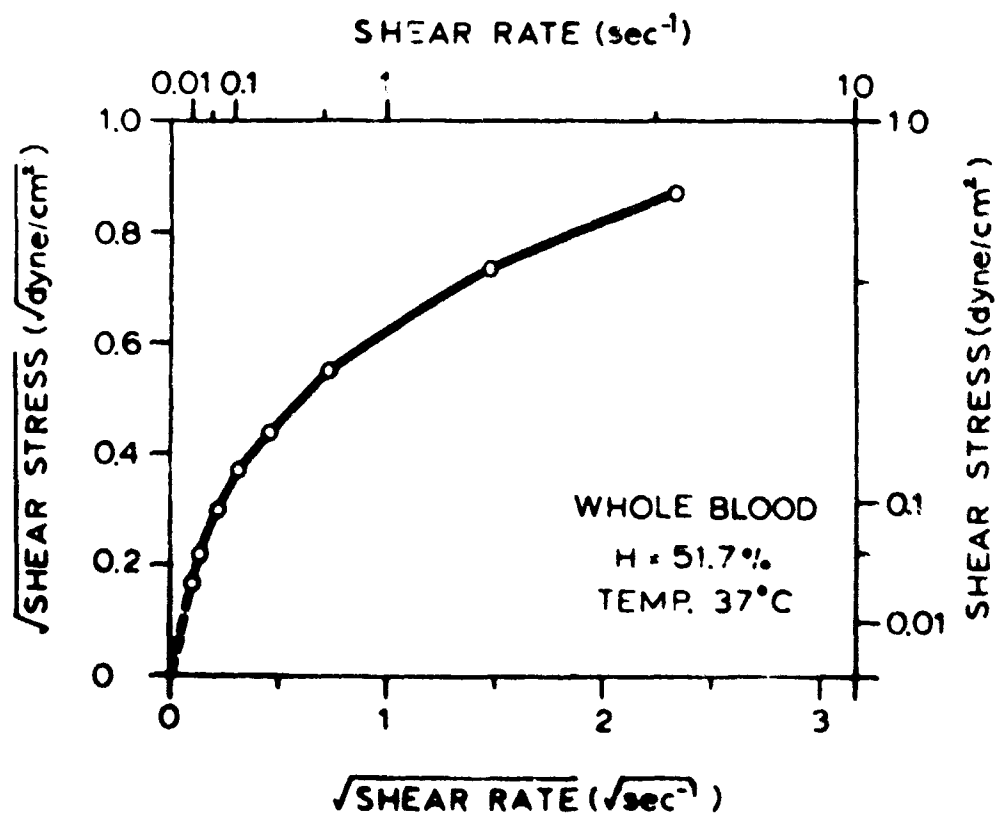


Fig. 9. Relationship between the square root of shear stress and the square root of shear rate in a sample of whole blood (same sample as in Fig. 8). Note the ever-changing slope pointing to the (0, 0) origin.

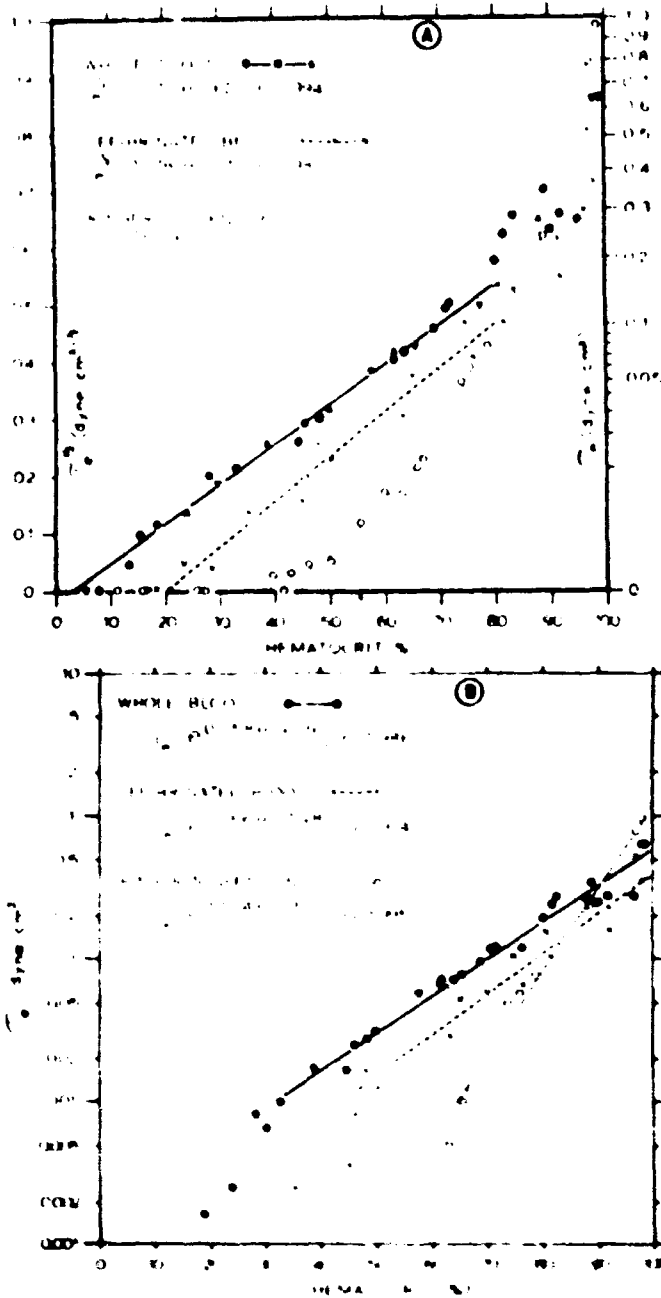
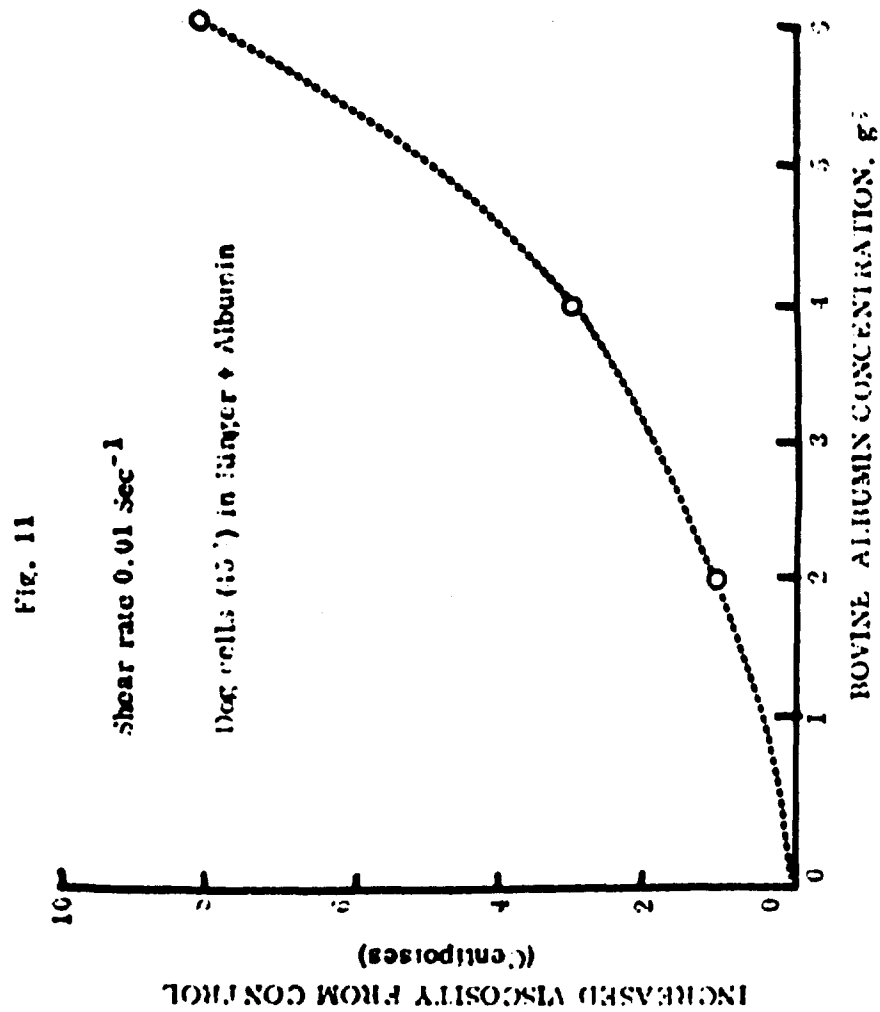
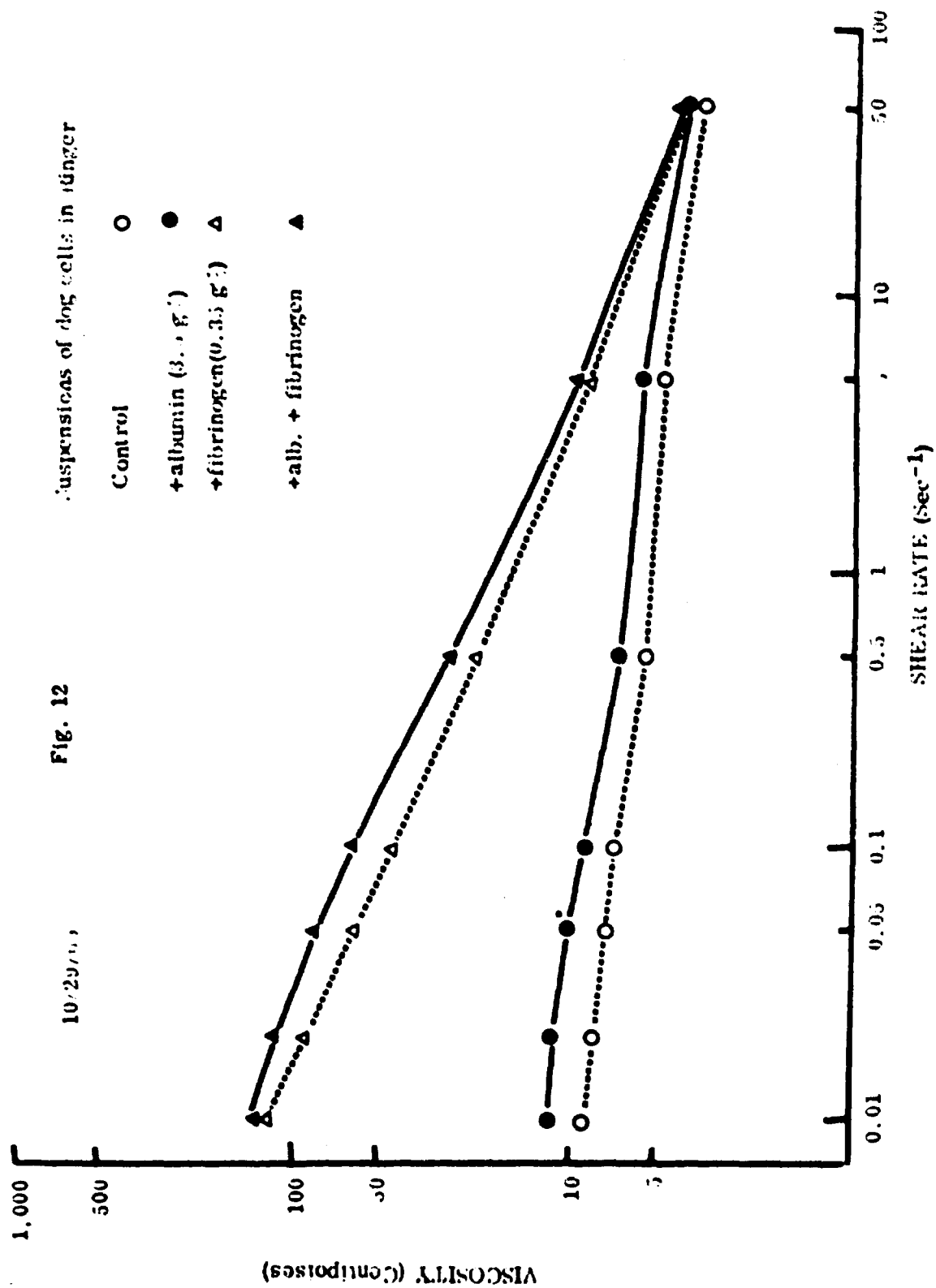


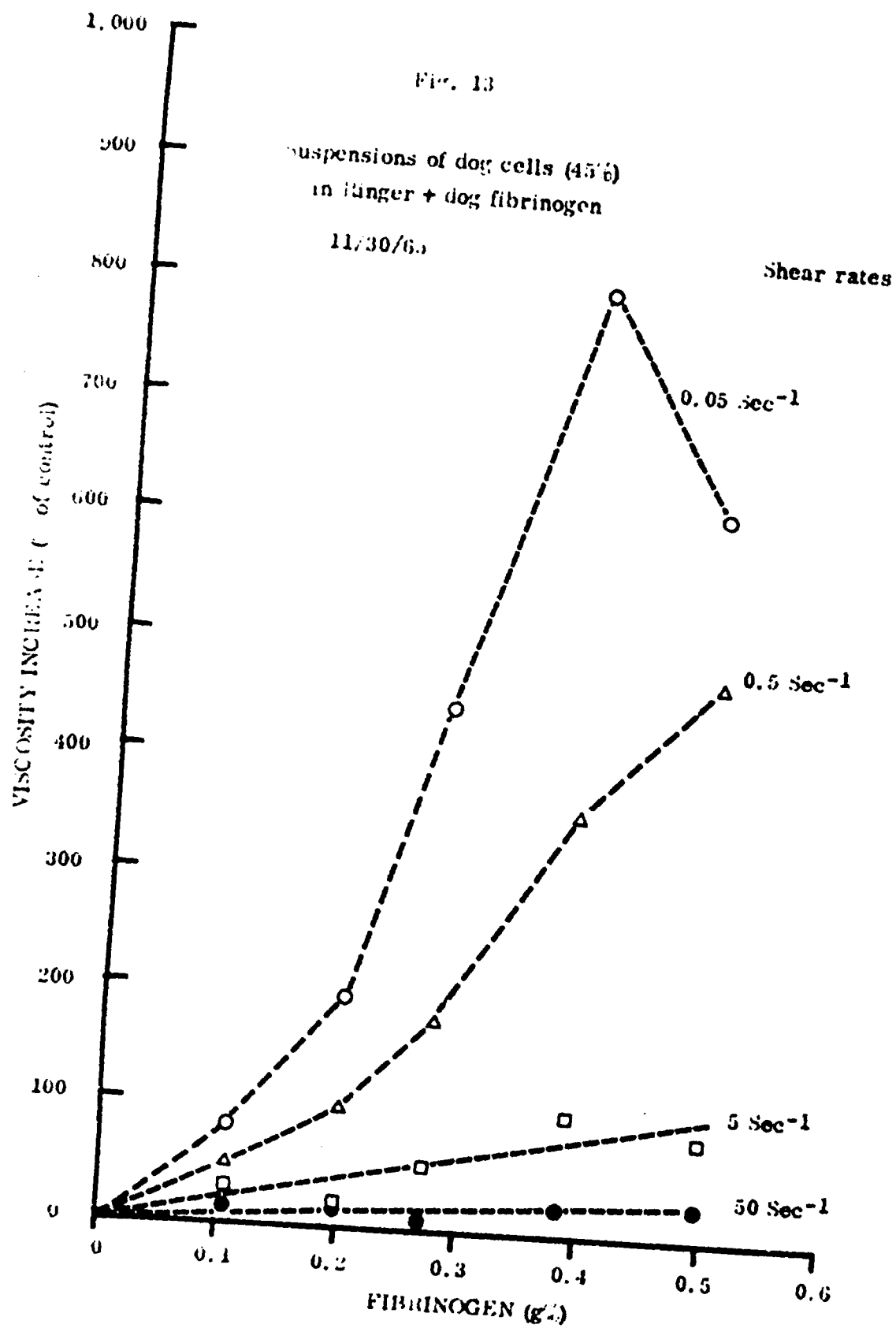
Fig. 10

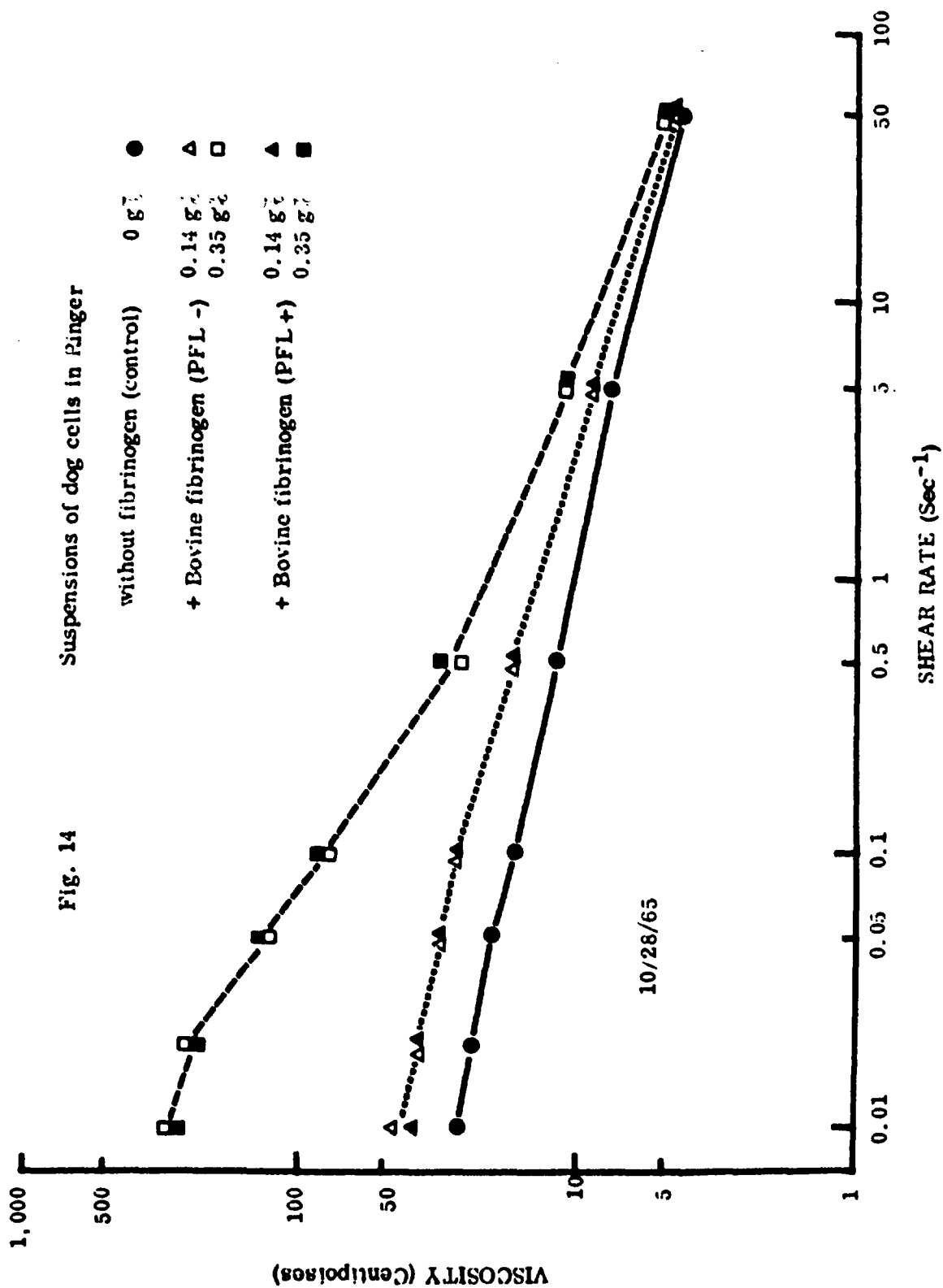
A. Relationship between the cube root of τ_e and the hematocrit. For whole blood, a different closed symbol is used for the results obtained on each of 5 subjects. The linear regression formulae and the coefficients of correlation (r) for whole blood and defibrinated blood (both up to 80% H) are given in the graph. The results on Ringer suspensions of cells show a curvilinear relationship.

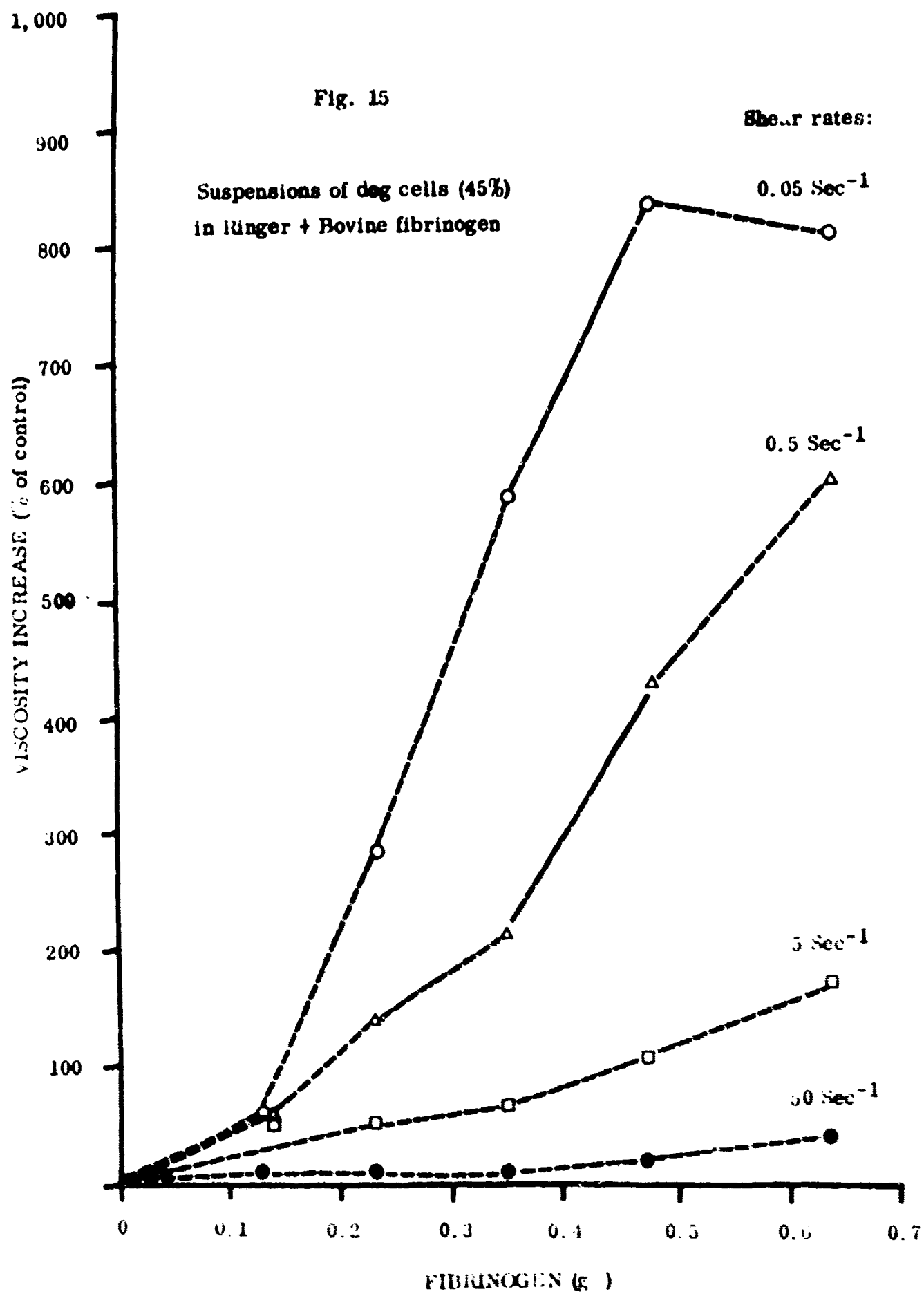
B. Relationship between the logarithm of τ_e and the hematocrit for whole blood, defibrinated blood and Ringer suspensions of cells. Note the linearity of the results when τ_e is higher than 0.01 dyne/cm² (whole blood H>32%, defibrinated blood H>47%, and Ringer suspension H>65%). The linear regression formulae and the coefficients of correlation (r) are given in the graph.











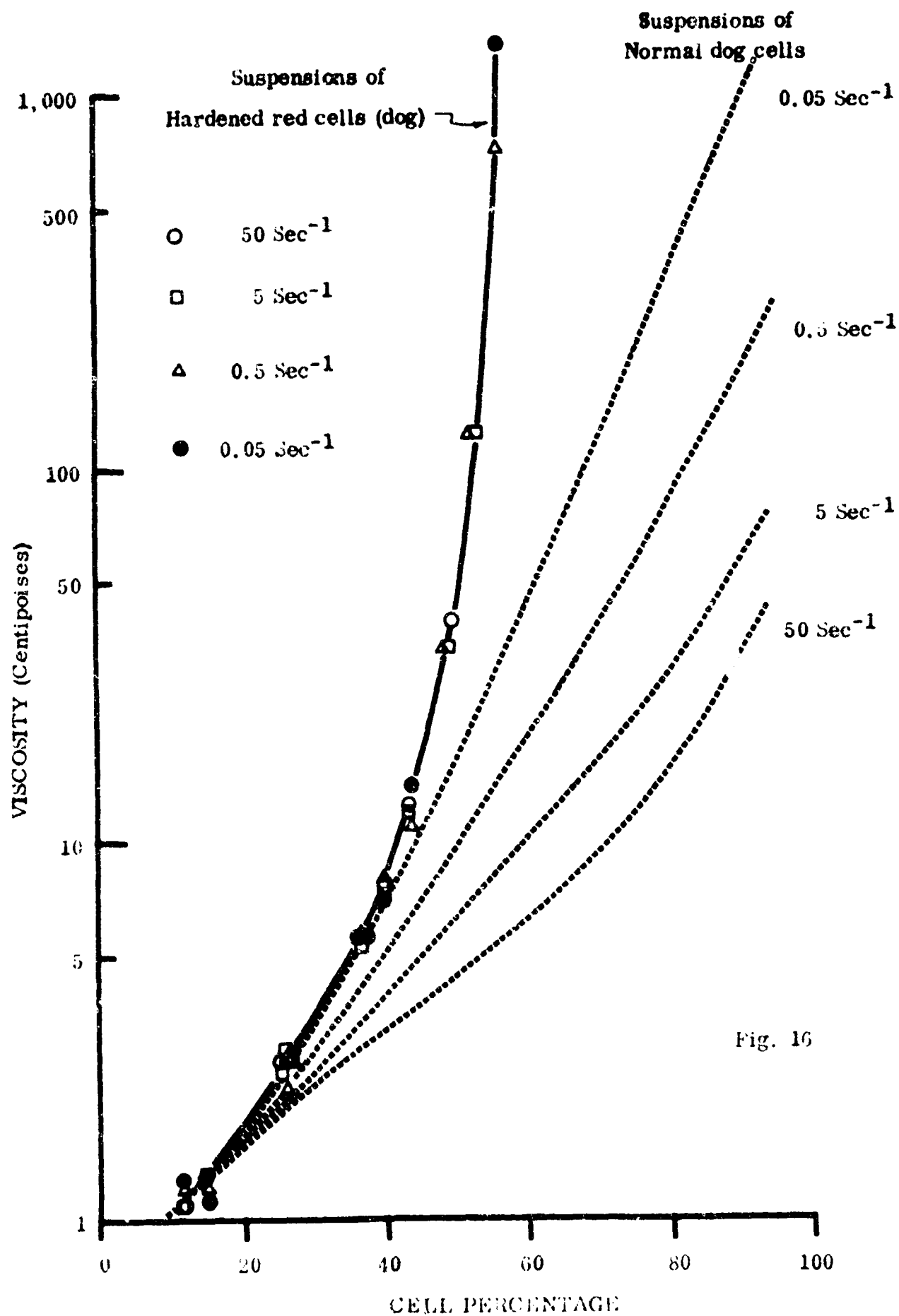
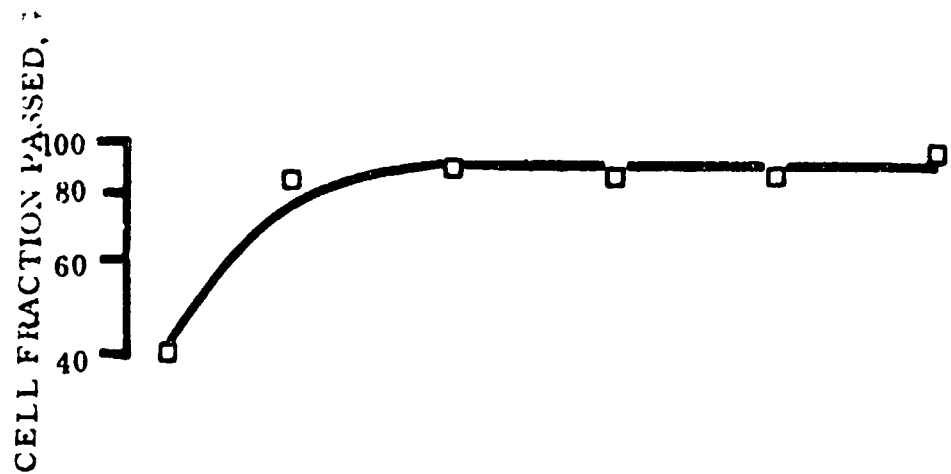
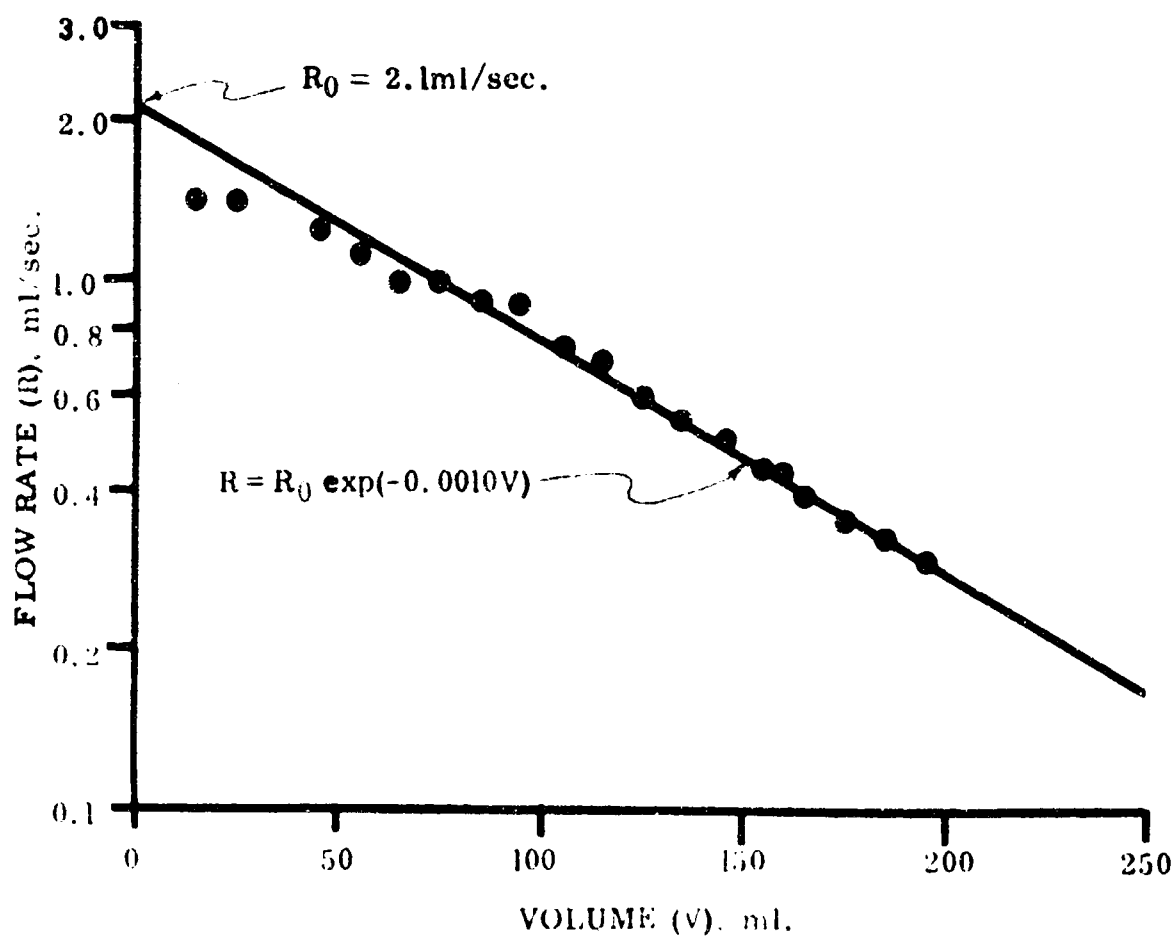


Fig. 16



25,000/mm³ washed dog erythrocytes through
silver filter of 5μ nominal pore size

Fig. 17



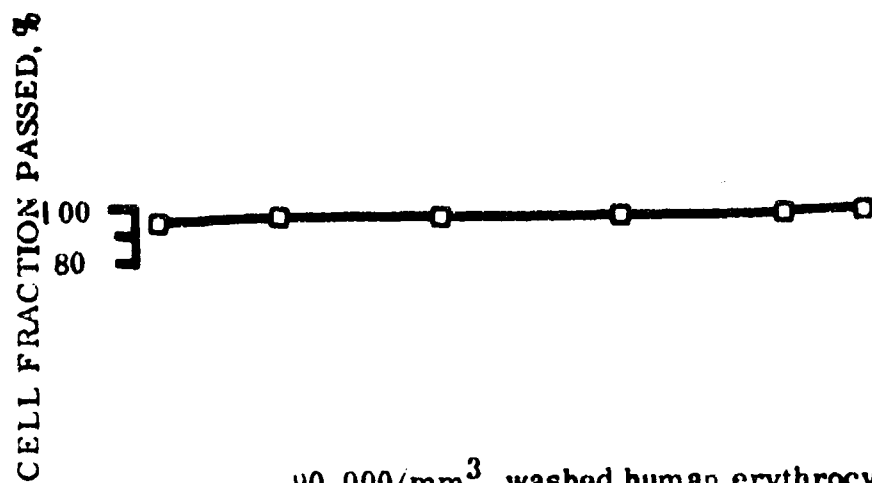
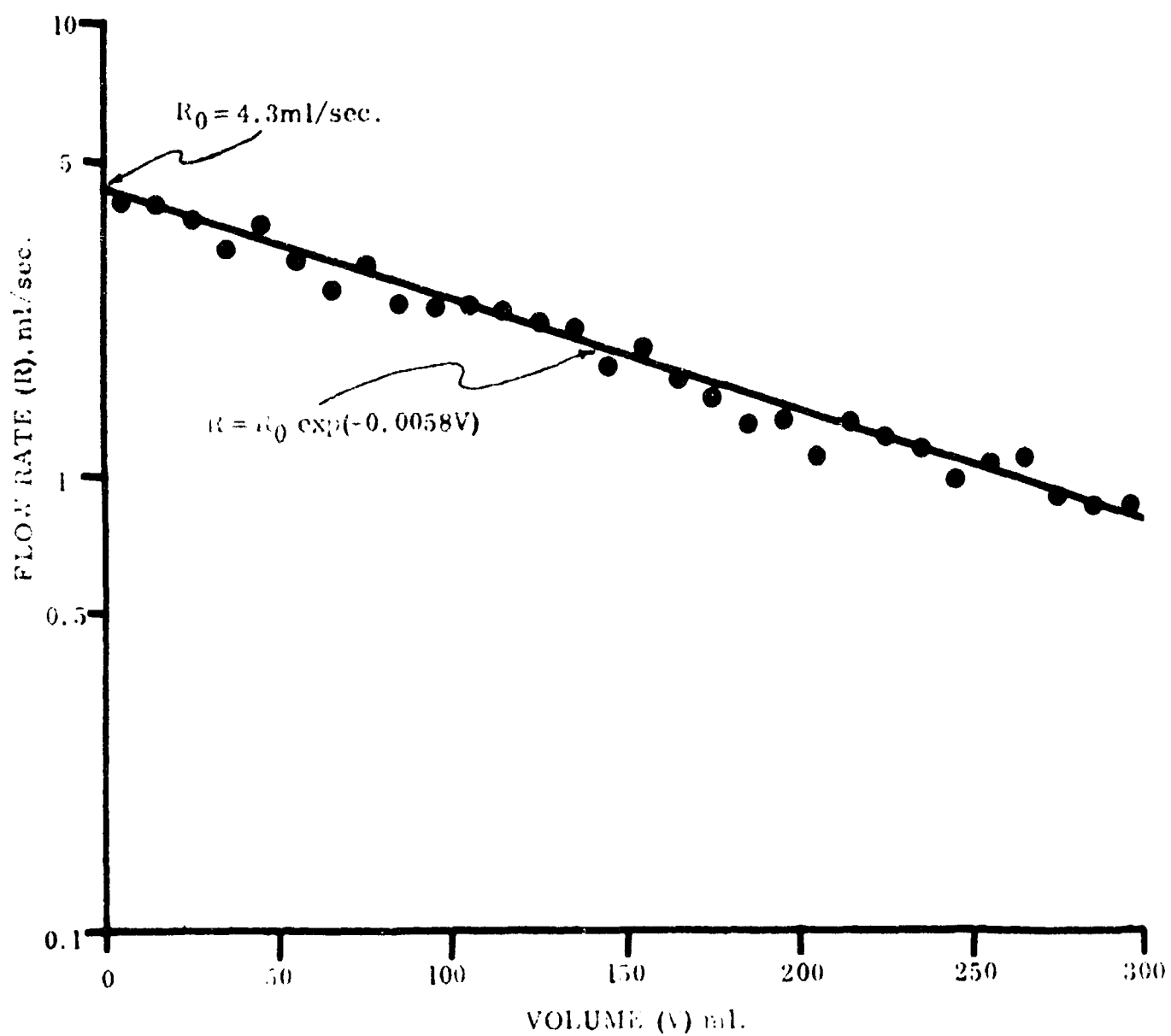


Fig. 18



ANNUAL PROGRESS REPORT-SECTION 2

1. Preparing Institution: Columbia University College of Physicians and Surgeons,
New York City
2. Title of Report: STUDIES ON BLOOD VISCOSITY AT LOW SHEAR RATES (SECTION 2)
3. Principal Investigator: Hannus I. Gredersen, Dalton Professor of Physiology
4. Number of pages and illustrations: Section 2 contains 24 pages, 4 tables
and 16 illustrations.
5. Contract Number: DA-49-193-MD-2272
6. Supported by: U.S. Army Medical Research and Development Command,
Department of the Army, Washington, D.C.
7. Abstract (see Section 1).

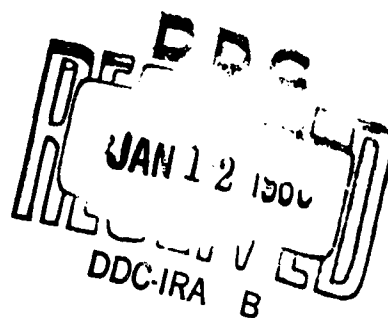


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II. EFFECTS OF ENDOTOXIN ON BLOOD VISCOSITY AND OTHER HEMODYNAMIC FUNCTIONS

Previous experiments (1964 Progress Report) have shown that endotoxin shock in the dog is associated with a marked increase in capillary permeability and a loss of fluids from the circulation. Some preliminary experiments were also described in the 1964 Report to indicate that the fluid loss was associated with a decrease in the plasma volume and an increase in blood viscosity. In the past year these studies have been extended and completed. The purpose of these experiments was to correlate the changes in blood volume, blood flow and blood viscosity with the alterations in capillary permeability, with the hope that the fundamental disturbance in endotoxin shock can be elucidated and that an ideal therapeutic measure can be devised. The experiments were performed on healthy, mongrel dogs. A total of 50 dogs was used, of which 12 had been splenectomized and 3 had been sympathectomized and splenectomized. All operations had been done at least one month prior to the endotoxin experiments. The endotoxin experiments were performed under sodium pentobarbital anesthesia (30 mg/kg). The various hemodynamic measurements were made in the controlled state and repeated following the intravenous injection of Escherichia coli endotoxin (3 mg/kg).

A. Cardiac Output

The cardiac output was determined with an indicator dilution method (Hamilton, Handbook of Physiology, Section 2, Volume I, Chapter 17, 1962, Am. Physiol. Soc.) using indocyanine green. Figure 1 summarizes the effects of E. coli endotoxin on cardiac output and other parameters of the systemic circulation in six normal dogs (spleen intact) and ten splenectomized dogs. The injection of endotoxin resulted in a typical course of arterial hypotension. Within 5 minutes the arterial pressure decreased to less than 40% of the control and then rose slightly.

A secondary fall usually commenced 40 minutes following endotoxin and was less severe in the splenectomized dogs. The cardiac output underwent alterations similar to those seen for the arterial pressure. The initial decrease in cardiac output, however, was more severe than the reduction in the arterial pressure and as a result, the calculated total peripheral resistance ($TPR = \text{arterial pressure} / \text{cardiac output}$) was approximately doubled during the first few post-endotoxin minutes. The partial recovery of arterial pressure between 5 and 40 minutes was accompanied by increases in both cardiac output and TPR. A secondary decrease in cardiac output was prominent in the dogs with spleen but was almost absent in the splenectomized dogs. In both groups of animals TPR rose progressively between one and three hours after endotoxin and the values were higher in the dogs with spleen. The heart rate decreased significantly during the first 20 minutes and then returned toward the control level. The stroke volume followed essentially the same time course as the cardiac output. Following endotoxin injection the arterial cell percentage (hematocrit corrected for plasma trapping) became significantly elevated in the dogs with spleen but not in the splenectomized dogs.

Figure 2 shows the results of arterial pressure and the cardiac output on two sympathectomized-splenectomized dogs. The control arterial pressure and the cardiac output in these two animals were lower than those observed in the splenectomized dogs with sympathetic system intact (Fig. 1). Following the injection of endotoxin, these measurements decreased also in the sympathectomized animals. After the first post-endotoxin hour, the cardiac output and the arterial pressure decreased by a greater percentage than in the splenectomized dogs with sympathetic system intact. The late rise in TPR seen in the dogs with intact sympathetics was not observed in the sympathectomized animals.

B. Venous Pressures

Venous pressures were measured in the left hepatic vein (catheterized under fluoroscopic control), the portal vein, the inferior vena cava (below the diaphragm), the superior vena cava (at its junction with the right atrium), and the left innominate vein. Fig. 3 illustrates the average results on venous pressures determined in the dogs with spleen. Endotoxin caused a marked rise in portal venous pressure. The hepatic venous pressure showed inconsistent changes depending upon the position of the tip of the catheter. When the catheter was wedged in the small hepatic veins the pressure change was similar to that of the portal venous pressure. When the catheter was placed more centrally, the pressure rise became progressively smaller. In experiments where the tip of the hepatic venous catheter was placed sufficiently centrally, the pressure decreased rather than increased after endotoxin injection. The increases in portal and wedged hepatic venous pressures usually reached peak values at 2 minutes and returned to control levels within approximately 20 minutes. The inferior vena caval pressure increased slightly at 5 minutes following endotoxin. The pressures recorded from the left innominate vein and the superior vena cava were closely similar and therefore grouped together in Fig. 3. Both pressures decreased significantly following endotoxin and remained low throughout the period of study. The changes in venous pressures in splenectomized dogs were essentially the same as those shown in Fig. 3 for the dogs with spleen intact. The marked elevation of portal venous pressure seen during the first few minutes after endotoxin was also observed in the dog that had been sympathectomized and splenectomized (Fig. 4).

C. Blood Viscosity

The viscosity of heparinized blood samples was determined in the modi-

fied GDM viscometer at a temperature of 37°C . The results on a normal dog and a splenectomized dog are compared in Fig. 5. In the normal dog with intact spleen, the increase in arterial cell percentage following endotoxin injection was accompanied by a rise in blood viscosity. The plasma protein concentration dropped after endotoxin but rose later. When the post-endotoxin results were compared with the viscosity-cell percentage relationship determined in the control state, the rise in viscosity was close to that expected from the increase in arterial cell percentage (Fig. 6). Actually, the first few post-endotoxin samples tended to have lower viscosity than the control samples adjusted to comparable cell percentages (Fig. 6), and this small difference can be attributed to the decrease in plasma protein concentration seen in the early post-endotoxin period (Fig. 5). In the splenectomized dogs (Fig. 5), the arterial cell percentage did not rise and the blood viscosity showed a slight decrease together with the plasma protein concentration. Table I summarizes the viscosity changes in five normal dogs at 5 minutes, 1 hour and 2 hours after endotoxin. A close correlation between the changes in viscosity and arterial cell percentage is evident. Fig. 5 also shows some correlation between the changes in blood viscosity and the total peripheral resistance, especially in the late stage of endotoxin shock in the normal dogs.

D. Total Blood Volume

The total blood volume was determined as the sum of the cell volume and the plasma volume, both of which were measured directly. The cell volume was determined with erythrocytes labeled with Cr^{51} . The plasma volume was determined with I^{131} -labeled albumin, T-1824, and dextran with a mean molecular weight of 250,000. Following the intravenous injection of these test substances, blood samples were usually collected at 10 minute intervals for 50 minutes or longer. By

alternating the injections of different test substances for plasma volume measurements, it was possible to determine the plasma volume at short intervals without curtailing the time-concentration curve after each injection. Simultaneous determinations with different substances showed good agreements both before and after endotoxin.

1. Total Plasma Volume. In the dogs with spleen, the plasma volume decreased after endotoxin (Fig. 7). The decrease was considerable even after allowance had been made for the plasma removed in sampling. The reduction of plasma volume became progressively greater with time and the loss was significantly greater in the dogs that died within 200 minutes after endotoxin. In the splenectomized dogs, plasma volume did not decrease significantly following endotoxin (Fig. 1). When corrections were made for the plasma removed in sampling, the post-endotoxin plasma volume of splenectomized dogs was larger than expected. The total circulating plasma proteins, calculated as the product of the plasma volume and the plasma protein concentration, decreased in all dogs after endotoxin. The decrease in splenectomized dogs was attributable to the lowering of the plasma protein concentration; whereas in the dogs with spleen it was due also in part to a reduction in plasma volume. In the non-survivals the larger reduction in plasma volume was accompanied by a higher plasma protein concentration. Two of the three sympathectomized-splenectomized dogs survived for longer than 200 minutes, and one died within this period. The changes in plasma volume in the two survivals (dogs B and C in Table II) were essentially the same as the splenectomized dogs with intact sympathetics, i.e. the post-endotoxin plasma volume was slightly larger than that expected from the control value minus sampling loss. The sympathectomized-splenectomized dog that died 140 minutes after endotoxin (dog A, Table II) showed a reduction in plasma volume greater than that expected from sam-

ling loss. The total circulating plasma proteins decreased in all three dogs, especially in the dog that succumbed at 140 minutes.

2. Total Cell Volume. In the dogs with spleen the average total cell volume decreased only slightly after endotoxin (Fig. 7). When corrections were made for the cells removed in sampling, the cell volume was found to increase in some experiments. Such increase in cell volume was associated with a rise in arterial cell percentage and a decrease in Cr^{51} activity per unit volume of erythrocytes (Fig. 8). In the experiments where there was no increase in cell volume, Cr^{51} activity per unit volume of erythrocytes remained constant even though the arterial cell percentage increased. In the splenectomized dogs, the decrease in cell volume was greater than that found in the dogs with spleen (Fig. 7). Even after the correction of sampling loss, there was still an average decrease of approximately 6% of the control cell volume. In the sympathectomized-splenectomized dogs, the post-endotoxin cell volume agreed well with that expected from control cell volume minus sampling loss. As in the splenectomized dogs, the arterial cell percentage did not rise and the Cr^{51} activity per unit volume of erythrocytes did not decrease.

3. Total Blood Volume and F_{cells} Factor. The total blood volume decreased progressively after endotoxin (Fig. 9), even after correction for blood sampling. Two and one-half hours after endotoxin, the measured blood volume decreased to an average of 73% of control. The dogs that did not survive within 200 minutes had lower blood volumes. Since the plasma volume and the cell volume were determined simultaneously, the overall cell percentage can be calculated. The ratio of the overall cell percentage to the arterial cell percentage, i.e. the F_{cells} factor, averaged close to unity in the control state (Table III). The measurements made approximately 80 minutes after endotoxin showed a significant decrease to an aver-

age value of 0.90. In the splenectomized dogs, the total blood volume did not decrease when corrections had been made for the amount of blood taken in sampling. The F_{cells} value averaged 0.88 in the control period and showed a significant decrease to an average value of 0.83 eighty minutes following endotoxin (Table III). The total blood volume in the two sympathectomized-splenectomized dogs that survived for longer than 200 minutes showed no decrease when correction was made for sampling loss, whereas it decreased slightly in the dog that died within this period (Table II). The F_{cells} factor showed the same decrease as in the splenectomized dogs with intact sympathetics.

E. Regional Blood Volume

1. Central Blood Volume. The central blood volume was calculated as the product of the central mean transit time and the cardiac output, both of which were determined from the dye dilution curve (Zierler, Handbook of Physiology, Section 2, Vol. I, Ch. 18, 1962, Am. Physiol. Soc.). In both the dogs with spleen and the splenectomized dogs, the central blood volume decreased within 5 minutes of endotoxin injection to approximately 60% of the control (Fig. 9). Between 5 and 40 minutes the central blood volume rose to about 70% of the control and then declined once again. The secondary decrease was more marked in the dogs with spleen. These findings on central blood volume followed closely the pattern of cardiac output and arterial pressure (Fig. 1). The central blood volume in sympathectomized-splenectomized dogs showed a greater decrease than in the dogs with intact sympathetics (Fig. 10). In all three groups of animals studied, the central blood volume was reduced by a larger percentage than the total blood volume throughout the post-endotoxin period.

2. Splanchnic Blood Volume. The splanchnic blood volume was calculated as the product of the splanchnic mean transit time and the splanchnic blood flow.

Changes in blood viscosity and shear stress as the shear rate approaches zero are important for ascertaining whether or not blood can sustain a yield stress before flow begins. As depicted in Fig. 7, down to a shear rate of 0.052 sec^{-1} , the viscosity of whole blood at a normal hematocrit apparently showed a continually upward trend, suggesting the existence of a yield point. However, this suggestion was not supported by measurements made at still lower shear rates. When measurements were extended down to a shear rate of 0.01 sec^{-1} , the log viscosity-log shear rate plot gave a sigmoidal curve tending to reach plateaus at both ends of the shear rates studied (Fig. 3). Other investigators have found that, at sufficiently high shear rates (above $200\text{--}400 \text{ sec}^{-1}$), blood is similar to a Newtonian fluid with a constant, low viscosity (Cerny et al., Am. J. Physiol. 202:1188, 1962). The present experiments on shear rates down to 0.01 sec^{-1} suggest that at very low rates blood again approaches another Newtonian region with high viscosity. If such a second Newtonian region with a constant viscosity exists, then at zero shear rate the shear stress (viscosity x shear rate) also would be zero without a yield stress. In order to study the changes in shear stress as the shear rate approaches zero, rheological data on blood often have been presented in the form of the square root of shear stress ($\tau^{1/2}$) versus the square root of shear rate ($\dot{\gamma}^{1/2}$) (Casson, in Rheology of Dispersed Systems, ed. by C.C. Mills, 1959, Pergamon; Cokelet et al., Trans. Soc. Rheol. 7:303, 1963), a plot in which the low ends of the coordinates are mathematically expanded for detailed examination. If a linear relationship exists between these two variables, then

$$\tau^{1/2} = \tau_y^{1/2} + b^{1/2} \cdot \dot{\gamma}^{1/2} \quad (1)$$

vation of the portal venous pressure which preceded the beginning of the decrease in arterial pressure by a few seconds. The portal hypertension was accompanied by an increase in the wedged hepatic venous pressure and a decrease of the larger hepatic venous pressure, indicating that there was a constriction of the hepatic venules or small veins. Furthermore, the hepatic blood flow showed a marked reduction during this early stage. The hepatic venoconstriction began to subside approximately 2 minutes after endotoxin and essentially disappeared in 20 minutes, as evidenced by the return of the portal and the wedged hepatic venous pressures. At the same time, the hepatic blood flow also recovered partially. At the peak of hepatic venoconstriction, the pressure gradient between the sinusoids (as estimated from the wedged pressure) and the large hepatic veins increased 4-fold (Fig. 3) and the hepatic blood flow decreased to less than one-fifth of the control. Therefore the hepatic venous resistance must have increased to at least 20 times normal. Since the elevation in portal venous pressure was also observed in totally sympathectomized dogs (Fig. 4), sympathetico-adrenal activity cannot be a significant factor in causing the hepatic venoconstriction. It rather seems that the sympathetic-adrenal activity was a result of the arterial hypotension following the hepatic venoconstriction. Since TPR was elevated in the early post-endotoxin period, the decrease in arterial pressure was caused by the reduction in cardiac output. There is evidence that cardiac contractility is not impaired until the terminal stage of endotoxin shock (Alican et al., Am. J. Surg. 103:702, 1962). Therefore the decrease in cardiac output resulted primarily from a diminished venous return. Since the total blood volume measured in the early stage of endotoxin shock showed little change, even in the dogs with spleen, the decrease in venous return most likely was a result of venoconstriction in the liver and possible also elsewhere. As pointed out above, the elevation of TPR in the

early stage of endotoxin shock was seen in all three groups of dogs studied, including the sympathectomized animals. Therefore it was not dependent upon sympathico-adrenal activity. Furthermore, during this early stage blood viscosity did not increase, even in the dog with spleen. Hence the rise in TPR was probably due to (a) the marked venoconstriction in the liver and possibly also elsewhere and/or (b) the passive constriction of blood vessels (arterioles) as a result of the reduction of transmural pressure. In the early stage of endotoxin shock, the heart rate decreased significantly in both the normal dogs and in the splenectomized dogs with intact sympathetics. This bradycardia, however, was much less prominent in the sympathectomized animals. These findings suggest that endotoxin caused bradycardia primarily by a reduction in sympathetic impulses and only partially by an increase in vagal impulses to the heart.

In the early stage of endotoxin shock, the splanchnic blood volume usually increased. This is in agreement with the marked hepatic venoconstriction mentioned above. The hepatic venoconstriction, by increasing the outflow resistance from the splanchnic circulation, caused an expansion of the splanchnic blood volume and also a rise in the capillary hydrostatic pressure in the splanchnic organs. The latter change was responsible for the increase of the outward movement of fluids and proteins from the capillaries to the interstitial space, and lymph flow from the splanchnic region increased (1964 Progress Report). This loss of protein-rich fluid, however, was balanced by an influx of protein-poor fluid from the kidney and possibly also other sites (1964 Progress Report). Therefore the plasma protein concentration decreased significantly, but the total plasma volume remained essentially unchanged at this time. The slight sequestration of erythrocytes found in the splenectomized dogs was probably also due to the severe hepatic venoconstriction and stagnation of blood flow in the splanchnic region. In the

dogs with spleen, the sequestration of erythrocytes cannot be demonstrated because it was masked by the release from the spleen of red cells which had not yet been equilibrated with the injected Cr⁵¹-erythrocytes. In contrast to the expansion of splanchnic blood volume, the central blood volume showed a marked decrease, and there was a compensatory volume shift within the circulation. In the present experiments, the central venous pressure measured in the superior vena cava decreased indicating an adequate cardiac pumping. The slight and transient rise in the abdominal inferior vena caval pressure was attributable to a simultaneous increase in the intra-abdominal pressure. The latter was probably caused by the pooling of blood and fluid in the abdominal viscerae as well as by changes in respiratory movements.

G. Correlation of Hemodynamic Changes in the Late Stage of Endotoxin Shock.

As the hepatic venoconstriction subsided, the arterial pressure and cardiac output first returned toward normal but fell again approximately 40 minutes after endotoxin. Later than one hour following endotoxin injection, the arterial pressure was significantly lower in the normal than in the splenectomized dogs (Fig. 1). As the TPR was higher in the normal dogs, the lower arterial pressure was attributable to a smaller cardiac output. In the absence of any evidence of cardiac insufficiency or marked volume deficiency (Fig. 7) at this time, the reduction in cardiac output was apparently initiated by an impedance to venous return. At a time corresponding to the onset of the secondary decrease in cardiac output, an increased segmental resistance in the small mesenteric veins and the small veins in the legs has been demonstrated (Meyer and Visscher, Am. J. Physiol. 202:913, 1962; Hinshaw et al., Am. J. Physiol. 202:103, 1962). Such increases in postcapillary resistance can result from several factors. First, endotoxin

causes the release of a number of vasoactive substances, including histamine, which can cause constriction of small veins in the leg (Haddy, Am. J. Physiol. 198: 161, 1960). Second, sympathetico-adrenal discharge in endotoxin shock also can cause venoconstriction, especially as endotoxin shock progresses and vascular reactivity changes such that the postcapillary effect of catecholamines is enhanced and their precapillary action diminishes (Spink et al., Proc. Soc. Exper. Biol. Med. 112:795, 1963). Besides these humoral and neural influences on the veins, the postcapillary resistance may also rise as a result of changes in the flow properties of the blood. Thus intravascular coagulation may occur in the venules, blocking the outflow from the capillaries (Hardaway et al., Ann. Surg. 154:791, 1961). The coagulation process, once initiated, may constitute a vicious cycle, because the slowing of flow would further favor clotting.

Another change in flow property of blood is found in its viscosity. Since blood viscosity rises as the shear rate is reduced, a decrease in blood flow in endotoxin shock would lower the shear rate and hence raise the blood viscosity even if the composition of the blood remained unchanged. In dogs with spleen, the contraction of this organ caused a rise in hematocrit and this resulted in a rise of viscosity at any given shear rate (Fig. 6, Table 1). Therefore in these animals, the in vivo blood viscosity in the venules would rise for two reasons, namely the lowering of the shear rate and the increase of hematocrit. The rise in viscosity caused an increase in resistance to flow, especially in the postcapillary venules where the shear rate is low.

The above discussion indicates that the vascular and blood changes in the late stage of endotoxin shock can perpetuate themselves in vicious cycles, causing progressive increase in postcapillary resistance and reduction in venous return. The preferential increase in postcapillary resistance would favor the loss

of fluid from the capillaries. In the dogs with spleen, the fluid loss was evidenced by the progressive decrease of plasma volume time (Fig. 7). This plasma loss was not accompanied by a further lowering of the total circulating plasma proteins, indicating that a protein-poor fluid was lost from the circulation. The fluid loss aggravated the situation because it on the one hand increased the hematocrit and the viscosity and on the other hand it reduced the blood volume. It is interesting to note that the dogs that died within 200 minutes had a lower plasma volume and higher plasma protein concentration and hematocrit than the survivors. Furthermore the splenectomized dogs, which had a higher plasma volume and showed hemodilution, all survived for longer than 200 minutes. These results point to the importance of the progressive loss of protein-poor plasma fluid in causing a vicious cycle. The greater loss of plasma fluid in the dogs with spleen seems to indicate that this fluid loss was related to the higher blood cell percentage caused by splenic contraction and the resulting deleterious effects on the microcirculation. It is possible that the presence of the spleen can have other detrimental effects than the simple release of erythrocytes. It would be interesting to extend the present studies to other animals, e.g. monkeys, in which the spleen does not store a large amount of erythrocytes. In the dogs with spleen, although the volume reduction per se did not account entirely for the hemodynamic changes in endotoxin shock, it played a contributory role in the progressive deterioration of the circulation.

The preferential constriction of postcapillary vessels not only caused a reduction in total blood volume but also a redistribution of blood volume in the circulation. Thus when arterial pressure was lower than 50% of control, the splanchnic blood volume increased rather than decreased. This increase in splanchnic blood volume seems to be due to primarily the increase in blood volume in the in-

testine, since in the late stage intestinal venoconstriction has been demonstrated (Meyer & Visscher, Am. J. Physiol. 202:913, 1962), whereas hepatic venoconstriction had already subsided (Fig. 3). The absence of any marked increase of splanchnic blood volume in sympathectomized dogs at low arterial pressure (Table II) indicates that sympathetic venoconstriction played a role in causing the splanchnic pooling. The greater tendency for splanchnic pooling to occur in the dogs with the spleen suggests that increases in blood viscosity (especially in the postcapillary venules) contributed to this occurrence. In contrast to the increase of splanchnic blood volume in the late stage of endotoxin shock, the central blood volume remained low throughout the entire period of study. Furthermore the per cent decrease of central blood volume was always greater than per cent reduction in total blood volume, indicating that it was a compensatory process to increase the effective circulating blood volume and the venous return.

The late rise in TPR seen in the splenectomized dogs with intact sympathetic system was absent in the sympathectomized-splenectomized dogs, suggesting that in the splenectomized dogs sympathetic activity was the major reason for this late resistance rise. By comparing the TPR changes in the normal dogs, splenectomized dogs and the sympathectomized-splenectomized dogs (Fig. 1 & 2), it is seen that the late rise of TPR in normal dogs resulted principally from increases in hematocrit and viscosity and to a lesser extent from sympathetic vasoconstriction. During the 200 minutes of observation period after endotoxin, none of the 12 splenectomized dogs died, whereas one of three sympathectomized-splenectomized dogs did. This, together with the maintenance of the higher cardiac output in splenectomized dogs than the sympathectomized-splenectomized dogs (Fig. 2), seems to suggest that sympathetic activity was beneficial in the splenectomized dogs given endotoxin. The beneficial effect is most likely the result of the sympathetic influence on cardiac performance. Although sympathetic venoconstriction

may aggravate the vicious cycles leading to the deterioration of the microcirculation (Lillehei et al., Ann. Surg. 160:682, 1964), the detrimental effect was not apparent in the splenectomized dogs which did not show a rise in blood viscosity following endotoxin. In the normal dogs the elevations of hematocrit and viscosity already increased the resistance to flow, and therefore further sympathetic venoconstriction can cause excessive derangement of the microcirculation. The vicious cycles that lead to circulatory deterioration in the late stage of endotoxin shock are summarized in Fig. 12.

III. HEMODYNAMIC EFFECTS OF PERICARDIAL TAMPONADE

The hemodynamic changes produced by the introduction of a constant volume of saline into the pericardial cavity has been compared with similar changes produced by the maintenance of a constant fluid pressure within this cavity. Monorel dogs were operated upon at least 10 days prior to the experiment.* During the operation the spleen was removed and a catheter tied into the pericardial cavity. The catheter was exteriorized through the thoracic wall, sealed and sewn under the skin. On the day of the experiment this catheter was exposed under pentobarbital anesthesia, attached to a saline reservoir and fluid (saline at 37°C) was pumped into the space. The fluid volume administered was constant in 4 experiments and variable in the other 4 with an attempt to maintain a constant pericardial pressure. The hemodynamic changes resulting from these two procedures were recorded and compared.

A. Pericardial and Central Venous Pressure

During the control period, the pericardial pressure and central venous pressure were slightly below zero. With the onset of tamponade they increased to a positive value of approximately 13-14 cm saline. The pericardial pressure was generally slightly higher than the central venous pressure in both the constant pressure and constant volume series (Fig. 13). During the two hour period of the constant volume tamponade, both pericardial and central venous pressures decreased to near zero. In the constant pressure series such a decrease was not observed because of the further introduction of saline.

B. Arterial Pressure

As the pericardial and central venous pressures were elevated immediately following the onset of the tamponade, both arterial pressure and pulse pressure

* The collaboration of Dr. Robert T. Potter of the Dept. of Surgery, Brooklyn-Cumberland Hospital who did the surgical preparation of the dogs is gratefully acknowledged.

showed a marked reduction from the control values. The maintenance of constant pericardial and central venous pressures did not allow as much recovery of the arterial pressure as in the constant volume experiments where the arterial pressure returned almost to the control level during the two hour period of tamponade (Fig. 13). The pulse pressure showed only a limited recovery during constant pressure tamponade but increased markedly after the tamponade was released. This differs from constant volume tamponade where the pulse pressure gradually returned toward normal and did not show a marked increase following the removal of the saline.

C. Cardiac Output

The cardiac output decreased and total peripheral resistance increased at the onset of tamponade in both series of experiments. The constant volume experiments showed a continuous recovery of both the cardiac output and peripheral resistance during the tamponade period and both returned almost to control levels after the release of the tamponade. The constant pressure series showed almost no recovery of either cardiac output or total peripheral resistance during the two hour period, but following the release the cardiac output increased to 140% of control and the peripheral resistance decreased to 50% of control. During the subsequent hour these values gradually returned to control levels (Fig. 14). In both series of experiments the heart rate showed an initial drop, followed by a progressive increase attaining a maximum value of approximately 110-120% of control at the termination of the tamponade. The heart rate returned approximately to control values during the post tamponade period.

D. Oxygen Consumption

The oxygen consumption decreased by approximately 50% at the onset of the

tamponade in both the constant volume and constant pressure experiments (Fig. 14). In both series of experiments the oxygen consumption recovered to approximately 90% of the control value during the two hour period of the tamponade. Following release the control values were once again attained.

E. Plasma Protein Concentration and Hematocrit

Immediately after the onset of tamponade of both types, both the plasma protein concentration and the hematocrit began to decrease in a parallel manner, with the changes being greater in the constant pressure experiments. These changes were complete in approximately 30 minutes (Fig. 15). During the remainder of the tamponade period and also after the release, there was no significant change in hematocrit. There appears to be a slight recovery of the plasma protein concentration in the constant volume series during the remainder of the two hour period and also following release. This recovery is not evident in the constant pressure experiments except during the post tamponade period.

F. Total Blood Volume

The plasma volume, cell volume, total blood volume, total plasma proteins and overall F_{cells} factor are presented in Table IV where the control values are compared with those during the tamponade in both series of experiments. The total blood volume (ml/kg) increased by a greater degree in the constant pressure series than in the constant volume series and the increase can be attributed to the expansion of the plasma volume due to the hemodilution. The decrease in cell volume is accounted for by sampling loss. The total plasma proteins and overall F_{cells} factor both showed a decrease during the tamponade when compared to the control values.

G. Central Blood Volume

The central blood volume decreased to approximately 75% of the control value immediately following the onset of the tamponade (Fig. 16), but recovered somewhat in the constant volume experiments during the two hour period and also after release, but never returned to the control value. In the constant pressure experiments there was almost no recovery during the tamponade period, but following release the central blood volume increased to a value greater than the control and then gradually decreased during the remainder of the post tamponade period. Fig. 16 also shows the central mean circulation times, which increased in both series, the recovery being greater when the pericardial fluid volume was held constant. If the pressure was maintained constant, there was much less recovery during the two hour period, but a marked recovery to almost control values upon the release of the tamponade.

H. Comparison of Constant Volume and Constant Pressure Experiments

The increase in pericardial and central venous pressures, by the pumping of saline into the pericardial cavity, is sufficient to impede venous return to the right atrium and thereby produces a striking drop in cardiac output. In addition to the reduced venous flow there is a moderate reduction in heart rate during the first 15 minutes of the tamponade, which also contributes to the reduction in cardiac output. The reduced blood flow, despite the increase in total peripheral resistance, results in an immediate and marked drop in arterial pressure. This decrease activates the baroreceptor mechanisms producing an increase in heart rate which eventually exceeds the control values. The oxygen consumption drops initially because of the reduced blood flow but recovers during the course of the experiment almost to the control value. Since the recovery of oxygen consumption was

greater than that of the cardiac output, the oxygen extraction from the blood must have increased, especially in the constant pressure experiments. Coincident with the onset of the tamponade there is an increase in respiration rate and respiratory minute volume. A comparison of the two series of experiments shows that in the constant volume series, the pericardial and central venous pressures continuously and gradually decline over the period of the tamponade, also allowing a recovery of the reduced cardiac output by approximately 40%. As the cardiac output and arterial pressure recover, the total peripheral resistance also decreases progressively in the constant volume experiments. By contrast, in the constant pressure experiments the pericardial and central venous pressures remain elevated and the reduced cardiac output and the elevated total peripheral resistance do not change throughout the course of the experiment. The arterial pressure also recovers to a lesser extent in these experiments. The decrease in both pericardial and central venous pressures during the period of the tamponade may be explained by two possible causes. Firstly, there may be a leakage or absorption of fluid from the pericardial cavity, producing a reduced volume. Secondly, there may be a change in the size or shape of the heart or pericardium as an adaption to the fluid pumped into the cavity. However, with few exceptions, the volume recovered from the pericardium was less than the volume pumped into it, showing that some leakage or absorption must have occurred. This loss in volume could only be a partial explanation of the reduction in pericardial and central venous pressures, since the saline volume required to maintain the constant pressure was much larger than that unrecovered.

The reduced arterial pressure and the increased sympathetic activity probably facilitated entrance of fluid from the tissues which expanded the plasma volume and lowered the arterial hematocrit and plasma protein concentration. Therefore, in

pericardial tamponade, with the arterial pressure reduced and the venous pressure elevated, the capillary hydrostatic pressure apparently falls. The decrease in capillary pressure may be partially due to an increase in the pre-/post-capillary resistance ratio. A comparison of the two series of experiments indicates that the degree of hemodilution is greater in the constant pressure series (Fig. 15, Table IV), indicating that the capillary pressure is lower in these experiments. Since the elevation of total peripheral resistance is also higher in these experiments, it is suggested that the elevation of total peripheral resistance affects preferentially the precapillary segment. The fluid influx involves primarily a protein-free fluid since the total plasma proteins (gm/kg) do not increase in both series of experiments. The decrease in central blood volume in the face of a total blood volume greater than normal indicates that pericardial tamponade causes a redistribution of regional blood volume in the circulation.

IV. LIST OF PUBLICATIONS

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2. Chien, S., and S. Usami: Circulation of Sympathectomized Dogs Under Pentobarbital Anesthesia. Am. J. Physiol. 208:790-794, 1965.
3. Chien, S., R.J. Dellenback, S. Usami, and M.I. Gregersen: Hematocrit Changes in Endotoxin Shock. Proc. Soc. Exper. Biol. Med. 118:1182-1187, 1965.
4. Chien, S., R.J. Dellenback, and S. Usami: Effect of Endotoxin on the Transfer of Fibrinogen from Plasma to Lymph. Proc. Soc. Exper. Biol. Med. 118:1187-1190, 1965.
5. Taylor, H.M., S. Chien, M.I. Gregersen, and J.L. Lundberg: Comparison of Viscometric Behaviour of Suspensions of Polystyrene Latex and Human Blood Cells. Nature 207:77-78, 1965.
6. Gregersen, M.I., S. Chien, B. Peric, and H. Taylor: Investigations of Blood Viscosity at Low Rates of Shear: Effects of Variations in the Concentration and Character of the Red Cells and in the Composition of the Suspending Medium. Bibliotheca Anatomica 7:383-384, 1965.
7. Chien, S., S. Usami, R.J. Dellenback, K. Treitel, M. Barnett, and M.I. Gregersen: Effects of Endotoxin on Transfer of Plasma Proteins to Hepatic and Intestinal Lymph. Abstracts of Papers presented at XXIII Internat. Cong. Physiol. Sci., p. 148, 1965.
8. Gregersen, M.I., S. Usami, and S. Chien: Effects of Plasma Expanders on Viscosity of Human Blood. Abstracts of Papers presented at XXIII Internat. Cong. Physiol. Sci. p. 98, 1965.
9. Usami, S., S. Chien, R.J. Dellenback, and M.I. Gregersen: Species Differences of Blood Viscosity: Role of Cell Size and Suspending Medium. Physiologist 8:293, 1965.
10. Chien, S., R.J. Dellenback, S. Usami, and M.I. Gregersen: Plasma Trapping in Hematocrit Determination. Differences Among Animal Species. Proc. Soc. Exper. Biol. Med. 119:1155-1158, 1965.
11. Chien, S., R.J. Dellenback, S. Usami, and M.I. Gregersen: Capillary Permeability to Macromolecules in Endotoxin Shock. Third Conference on Artificial Colloidal Agents, National Academy of Sciences-National Research Council, pp. 56-73, March, 1965.
12. Gregersen, M.I., S. Chien, and S. Usami: Studies on the Effects of Hydroxyethyl Starch and Other Plasma Expanders on the Viscosity of Human Blood at Low Shear Rates. Third Conference on Artificial Colloidal Agents, National Academy of Sciences-National Research Council, pp. 29-35, March, 1965.
13. Gregersen, M.I., S. Usami, S. Chien, and P.L. Swank: Comparison of Screen Filtration Pressure and Low-Shear Viscosity of Blood. J. Appl. Physiol. 20:1362-1364, 1965.

TABLE I. EFFECTS OF ENDOTOXIN ON BLOOD VISCOSITY IN 5 NORMAL DOGS*

	Blood Viscosity (centipoises)				Arterial cell %
	Shear rate = 50 sec ⁻¹	5 sec ⁻¹	0.5 sec ⁻¹	0.05 sec ⁻¹	
Control	5.9±0.8	11.5±2.0	33±7	82±24	44.0±4.4
After endotoxin (change from control)					
5 min.	-0.6±0.2	-1.4±0.7	-6±3	-15± 5	-2.7±1.2
1 hr.	+2.5±0.6	+6.6±1.7	+25±7	+61±11	+12.7±3.5
2 hr.	+3.7±0.8	+9.6±2.1	+36±8	+103±19	+15.9±3.9

* Values are means ± S.E.M.

TABLE II. EFFECTS OF ENDOTOXIN ON BLOOD VOLUME IN SYMPATHECTOMIZED-SPLENECTOMIZED DOGS

Dog No.	Time (min.)	Total Blood Volume PV(ml)	CV(ml)	Art. cell %	(P.P.) (gm %)	Splanchn. BV(ml)	MAP (mm Hg)
A*	control	489	260	39.9	5.86	-	110
	40'	407	248	42.4	5.35	-	40
B	control	763	408	40.0	5.86	232	110
	17'	746	385	41.5	5.25	85	45
	90'	726	367	41.0	5.00	91	35
C	control	475	275	42.1	5.86	126	125
	45'	495	251	41.0	5.00	58	35
	110'	455	206	38.0	4.70	147	25

* Died 140 minutes after endotoxin

TABLE III. F_{cells} FACTOR IN ENDOTOXIN SHOCK

Dog No.	Control F_{cells}	Endotoxin F_{cells}	E - C
Dogs with spleen			
1	1.15	1.03	-0.12
2	0.97	0.84	-0.13
11	0.91	0.84	-0.02
12	0.94	0.93	-0.01
13	1.00	0.85	-0.15
19	1.06	0.91	-0.15
20	1.01	0.78	-0.23
21	1.02	0.94	-0.08
Mean	1.01	0.90	-0.11(P<0.01)
Splenectomized Dogs			
3	0.85	0.87	+0.02
4	0.86	0.82	-0.04
5	0.91	0.91	± 0.00
6	0.85	0.84	-0.01
9	0.86	0.81	-0.05
10	0.93	0.83	-0.10
22	0.88	0.87	-0.07
23	0.92	0.80	-0.08
24	0.93	0.80	-0.13
Mean	0.88	0.83	-0.05(P<0.01)

TABLE IV. EFFECTS OF PERICARDIAL TAMPONADE ON BLOOD VOLUME

	Constant Pressure (n = 4)		Constant Volume (n = 4)	
	Cont.	Tamp.	Cont.	Tamp.
Plasma volume (ml/kg)	48.3	54.2	47.5	50.3
Cell volume (ml/kg)	29.7	28.6	32.0	30.2
Blood volume (ml/kg)	78.0	82.8	79.5	80.5
Total plasma proteins (gm/kg)	2.99	2.82	2.76	2.56
F_{cells} factor	0.90	0.85	0.91	0.87

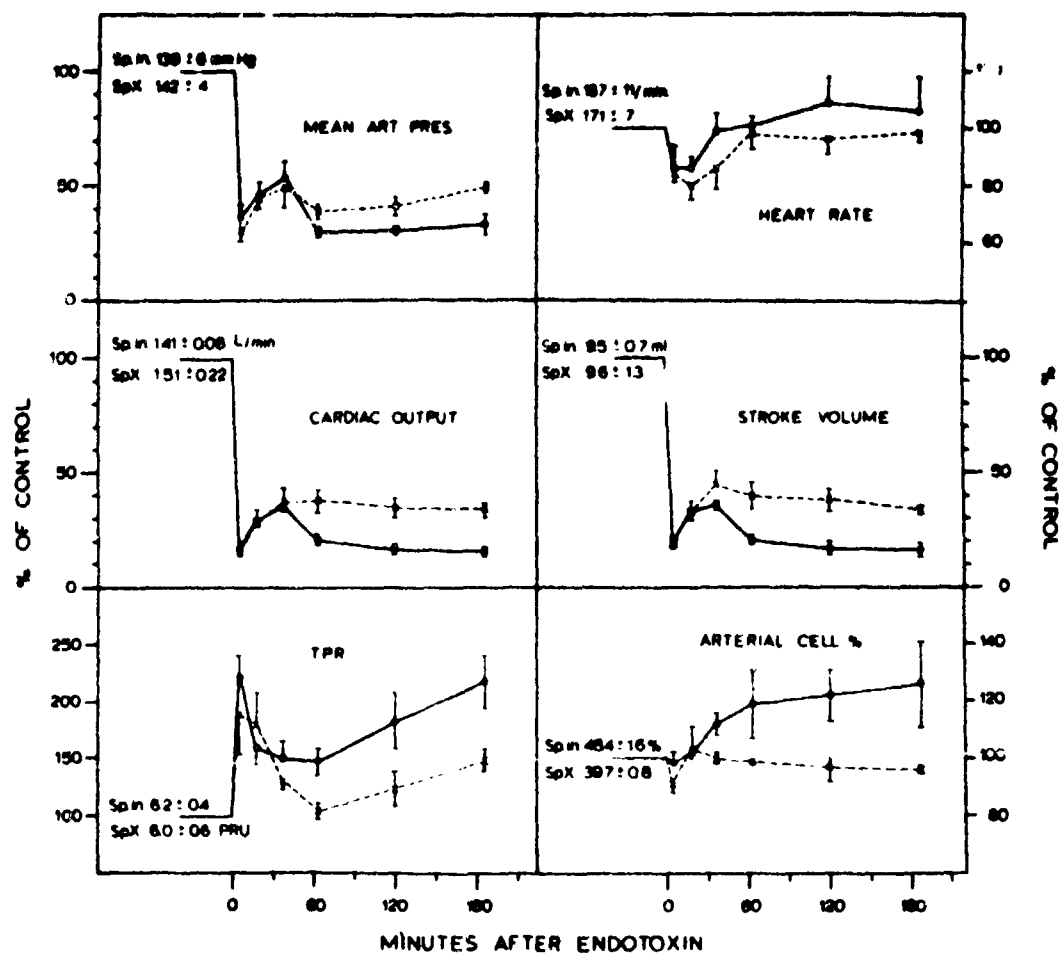


Fig. 1. Effects of endotoxin (given at zero time) on hemodynamic functions in 6 normal (Sp. in., circles) and 10 splenectomized (SpX, crosses) dogs. The numbers represent the means \pm S.E.M. for the control measurements. There is no significant difference between the control values of the two groups except the arterial cell percentage which is higher in the normal dogs (Student t test, $P < 0.01$). The post-endotoxin values are plotted as % of control and the vertical bars represent S.E.M. When the P value for the difference between normal dogs and splenectomized dogs is less than 0.05, closed circles are used for the results on normal dogs. Beginning at 30-60 minutes after endotoxin, the normal dogs had lower arterial pressure, cardiac output and stroke volume as well as higher TPR (total peripheral resistance) and arterial cell %.

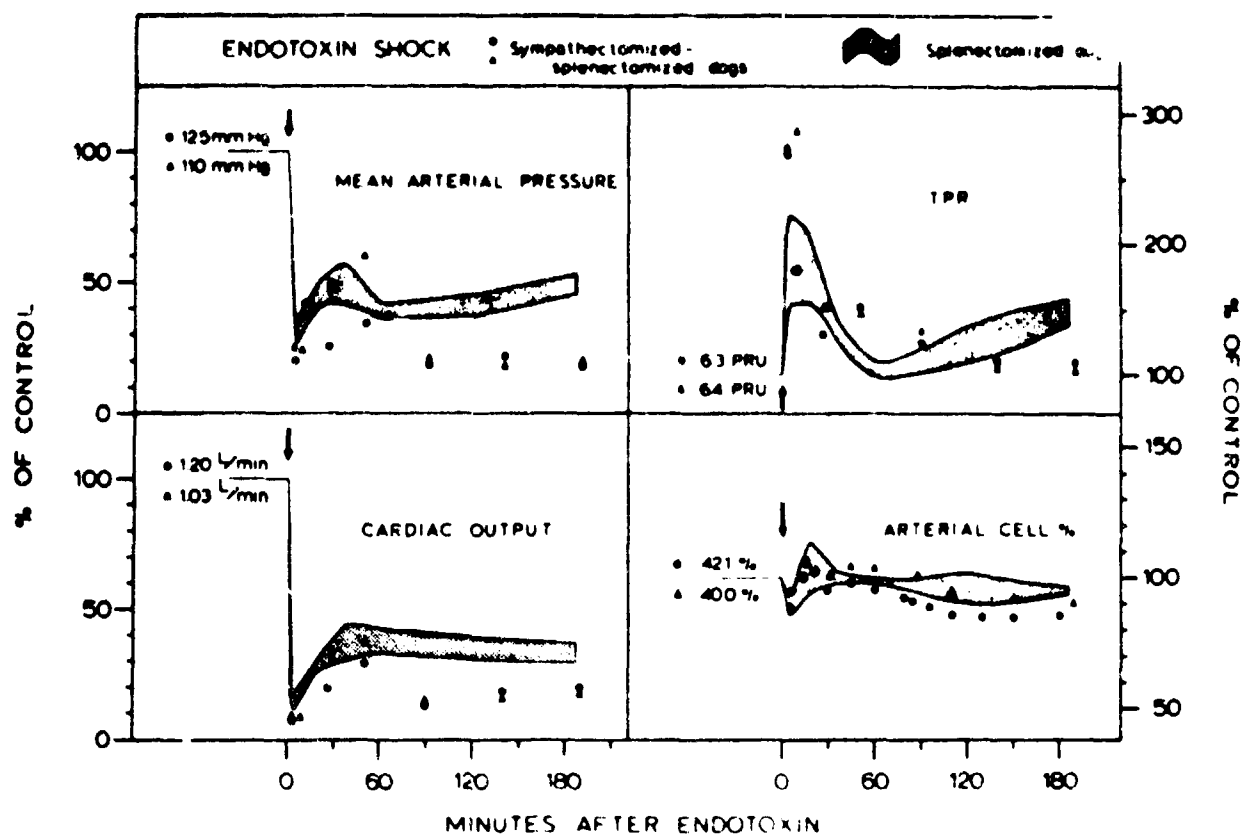


FIG. 2. Effects of endotoxin (given at arrows) on the hemodynamic functions of 2 sympathectomized-splenectomized dogs. The control values for these 2 dogs are given (cardiac output and TPR on a 10-kg basis). They had lower control arterial pressure and cardiac output than the splenectomized dogs with intact sympathetics (Fig. 1), but comparable control TPR and arterial cell %. The post-endotoxin changes seen in the splenectomized dogs (Fig. 1) are shown as shaded areas for comparison.

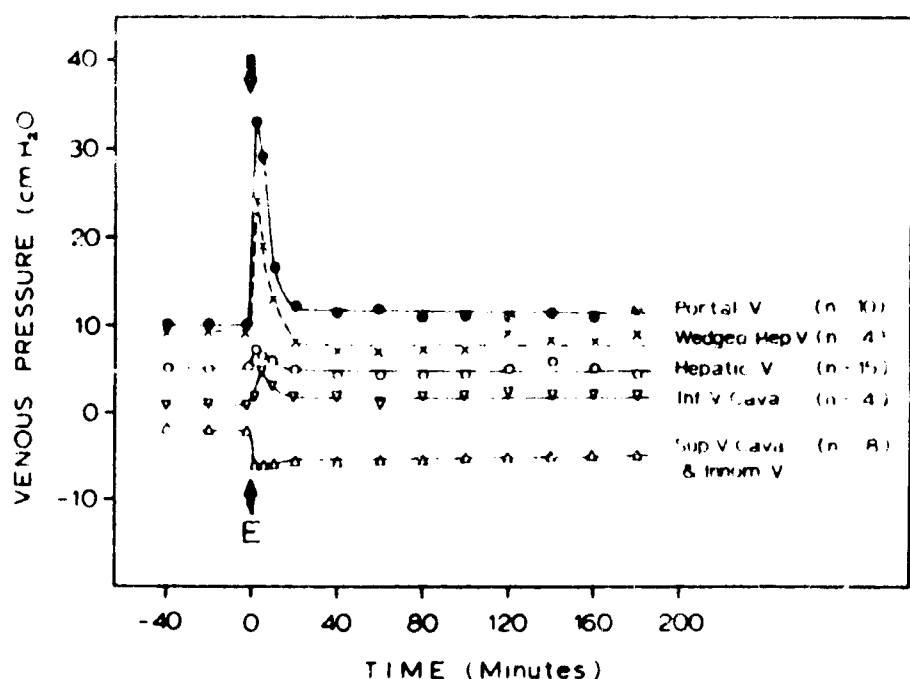


Fig. 3. Effects of endotoxin (E) on venous pressures in normal dogs. The number of dogs used in each measurement is given in parentheses. Note the parallel rises in portal venous and wedged hepatic venous pressure during the first few minutes after endotoxin.

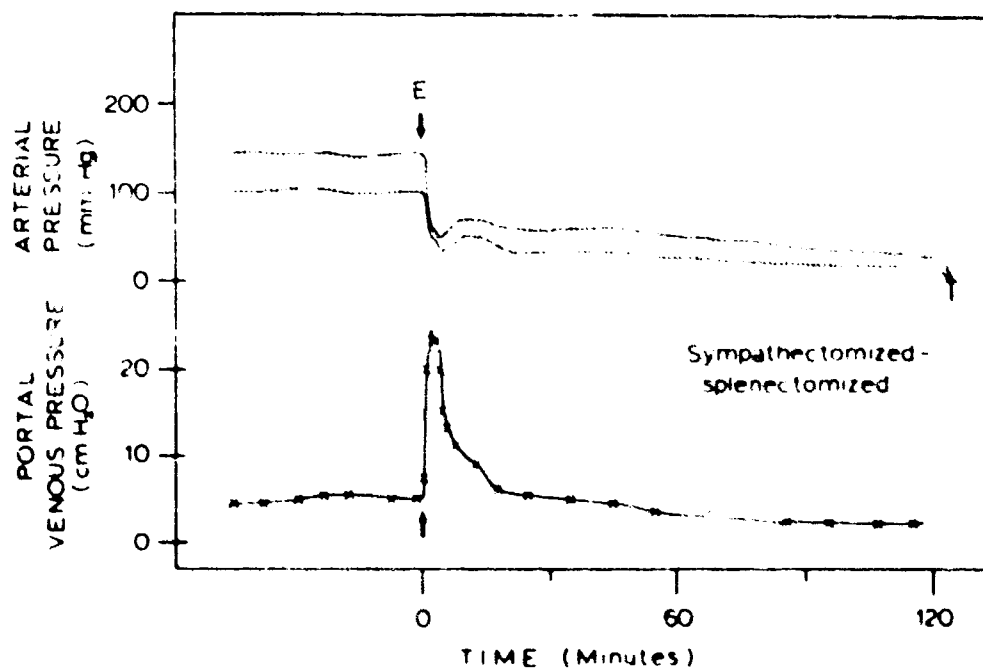


Fig. 4. Effects of endotoxin on the arterial pressure and the portal venous pressure in a sympathectomized-splenectomized dog. Note the similarity in portal hypertension between the sympathectomized dog and the dogs with intact sympathetics (Fig. 3).

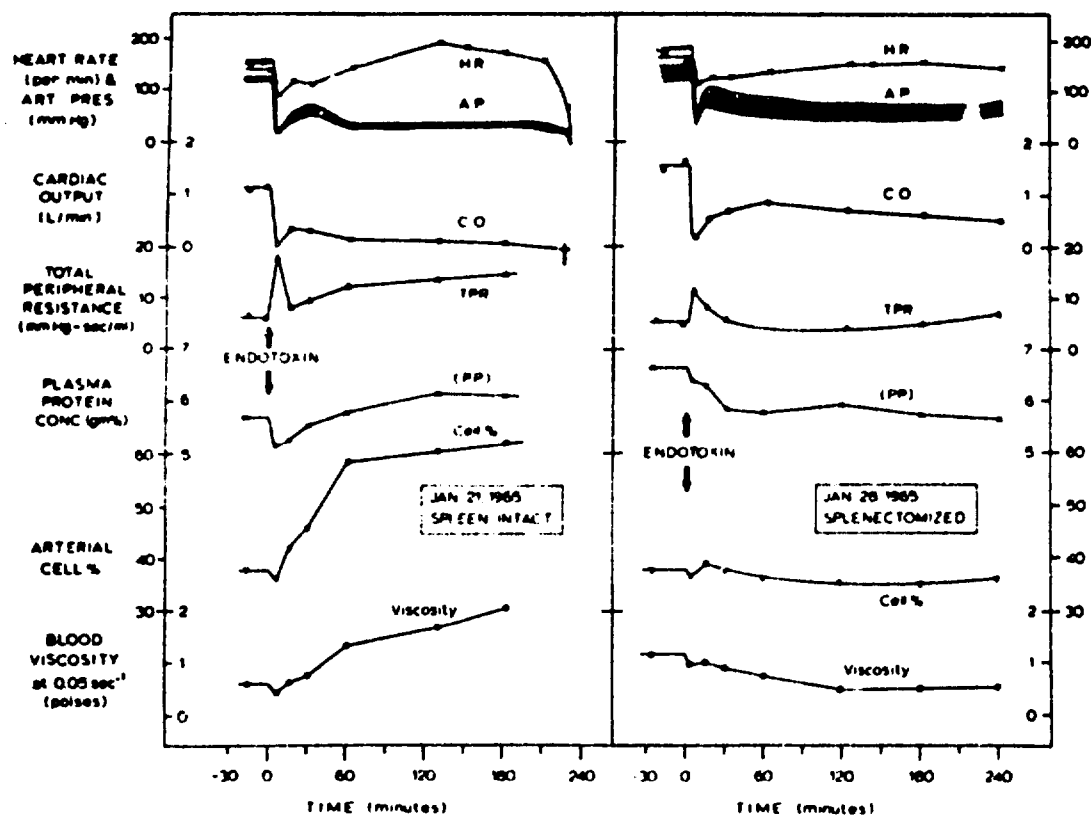


Fig. 5. Effects of endotoxin on blood viscosity (at a shear rate of 0.05 sec^{-1}) and other related measurements on a normal dog (spleen intact) and a splenectomized dog. Note the increase in blood viscosity in the normal dog and the decrease in the splenectomized dogs. These are accompanied by parallel changes in arterial cell % and plasma protein concentration. The secondary rise in TPR in the normal dog follows a time course parallel to that of the increase in viscosity. Poise, which is equal to 100 centipoises, is used as the unit for viscosity.

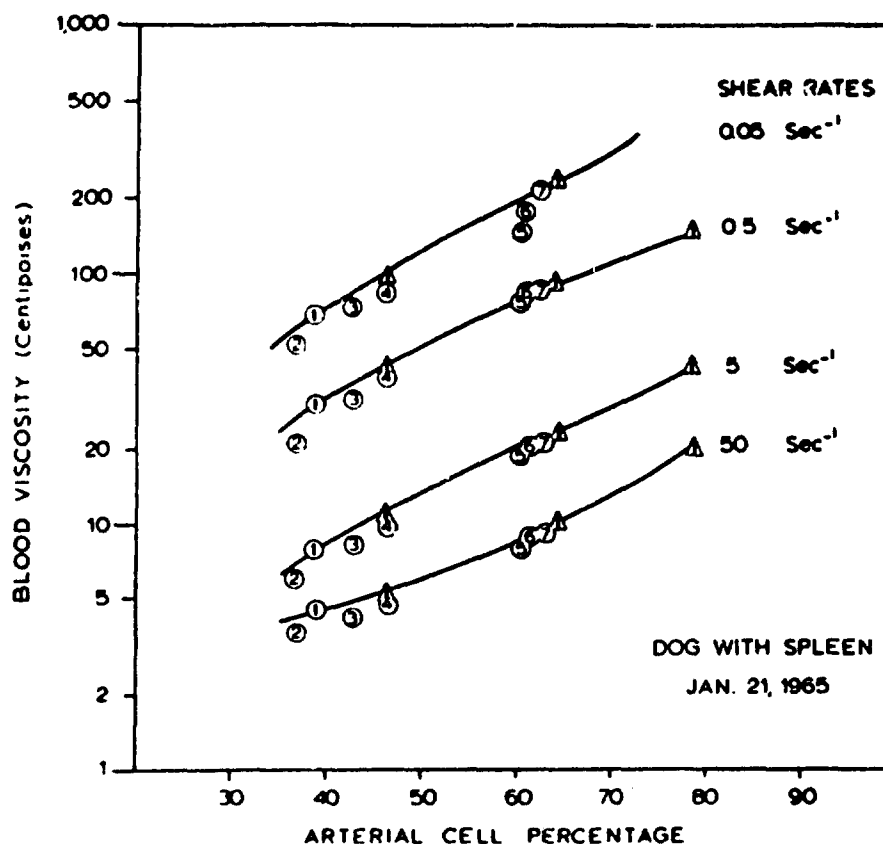


Fig. 6. Relation between blood viscosity and arterial cell percentage in a normal dog before (number 1) and after (2 to 7) endotoxin. For the control sample, viscosity was determined not only on the original blood (1 in circles) but also on samples readjusted to higher cell % by removing various amounts of plasma (1 in triangles). The values on these control measurements are then joined by lines which are curved and extended in manners according to the data obtained on a large number of experiments. As shown in Fig. 5, the blood viscosity increased progressively after endotoxin, and this Fig. indicates that the increase in viscosity can be accounted for on the basis of the rise in arterial cell %. In fact, the viscosity values on samples 2-5 fall slightly below that expected from their arterial cell %, and this can be attributed to the decrease in plasma protein concentration in these samples (Fig. 5).

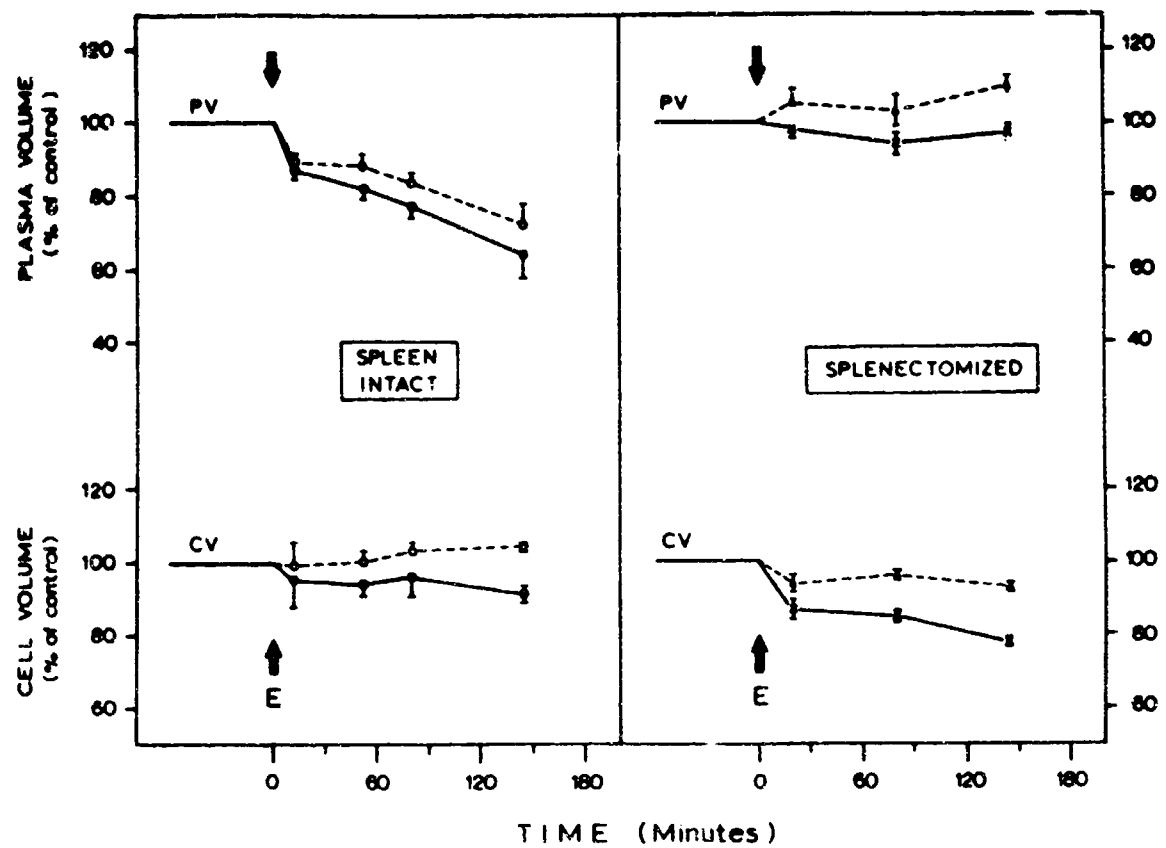


Fig. 7. Effects of endotoxin (E) on the plasma volume and cell volume in normal (spleen intact) and splenectomized dogs. Closed circles and crosses represent the measured values and open symbols depict the volumes corrected for sampling loss. The vertical bars denote S.E.M. Each point is the average of 4 to 17 determinations. Note the progressive reduction of plasma volume in normal dogs.

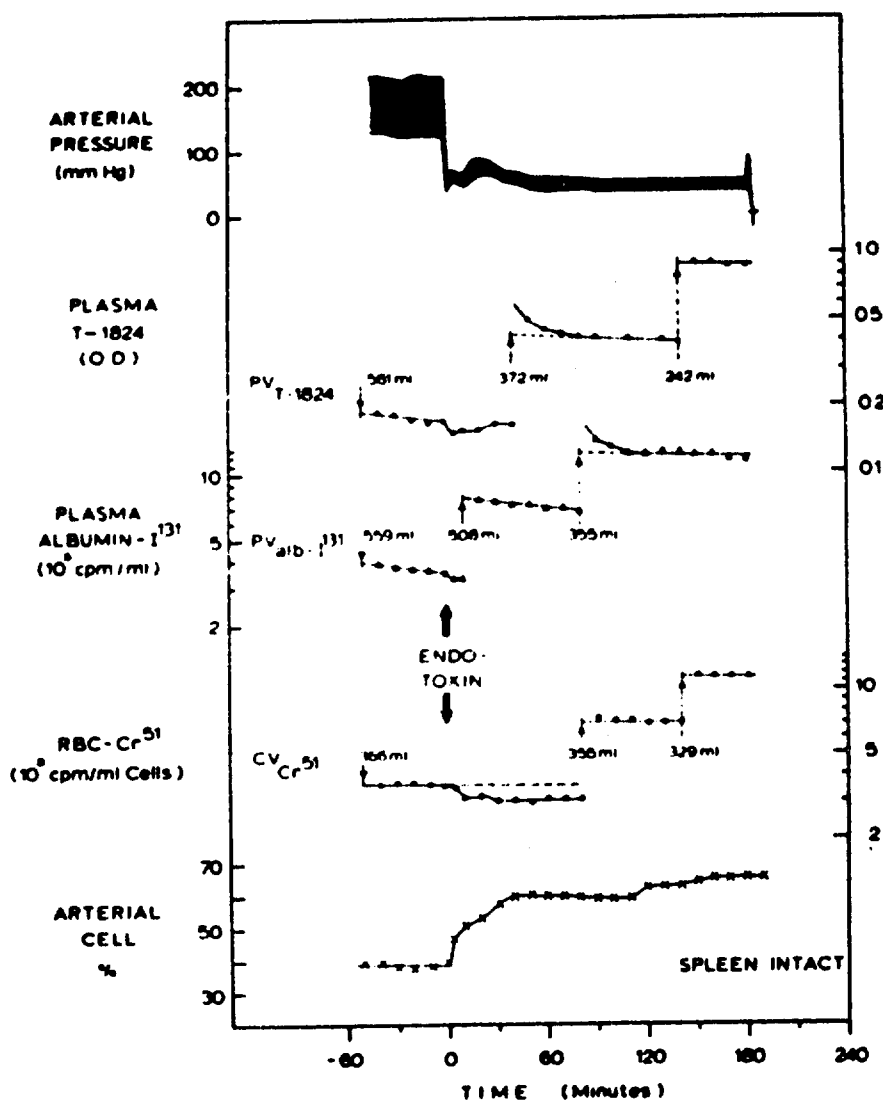


Fig. 8. Effects of endotoxin on plasma volume and cell volume in a normal dog. The thin arrows represent the time of injections of the test substances for plasma volume (T-1824 and albumin-¹³¹) and cell volume (RBC-Cr⁵¹) and the calculated volumes (not corrected for sampling) are given. Endotoxin results in an immediate rise in arterial cell percentage which is accompanied by a decrease in Cr⁵¹ activity per unit volume of RBC. The later rises in arterial cell percentage are associated with decreases in plasma volume. Note the delay in mixing of the test substances injected between 30 and 90 minutes after endotoxin.

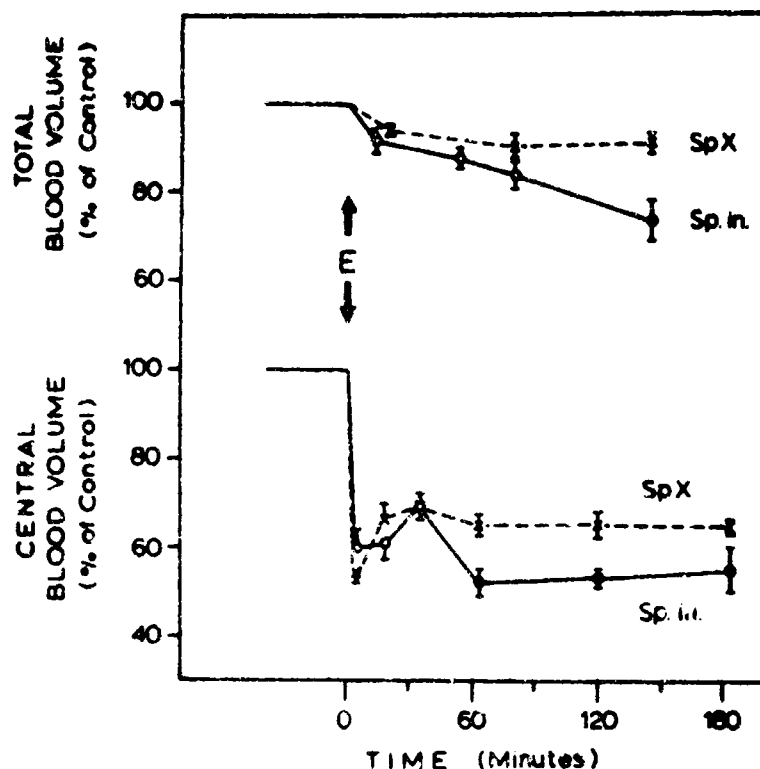


Fig. 9. Effects of endotoxin (E) on the total blood volume and central blood volume in splenectomized (spX, crosses) and normal (sp.in., circles) dogs. Vertical bars represent S.E.M. When the difference between the splenectomized dogs and normal dogs is significant at the 5% level (Student t test, $P < 0.05$), closed circles are used for the values obtained on normal dogs. Note the larger per cent decrease in central blood volume than total blood volume in both groups of animals.

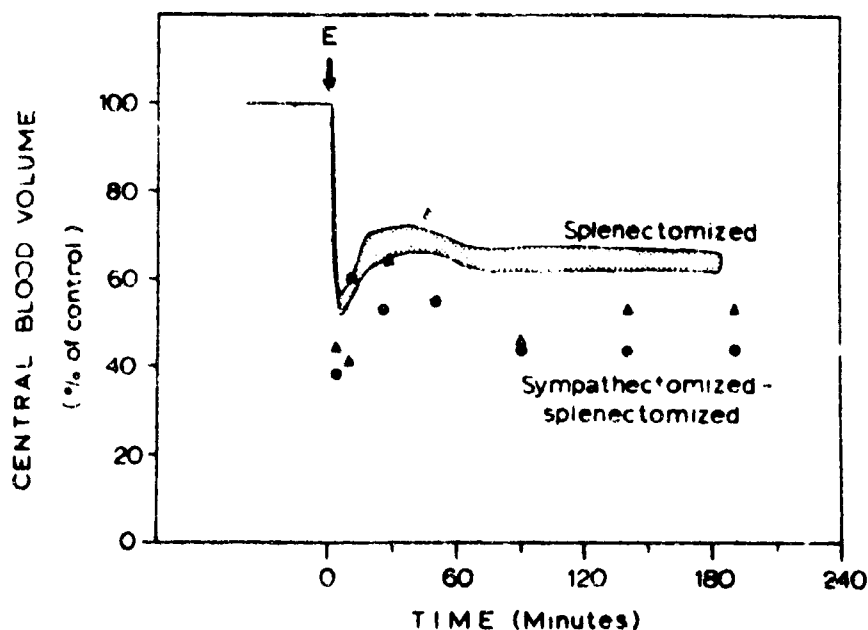


Fig. 10. Effects of endotoxin (E) on the central blood volume of 2 sympathectomized-splenectomized dogs. The shaded area represents the data (mean \pm S.E.M.) on splenectomized dogs (cf Fig. 3) and is included for comparison.

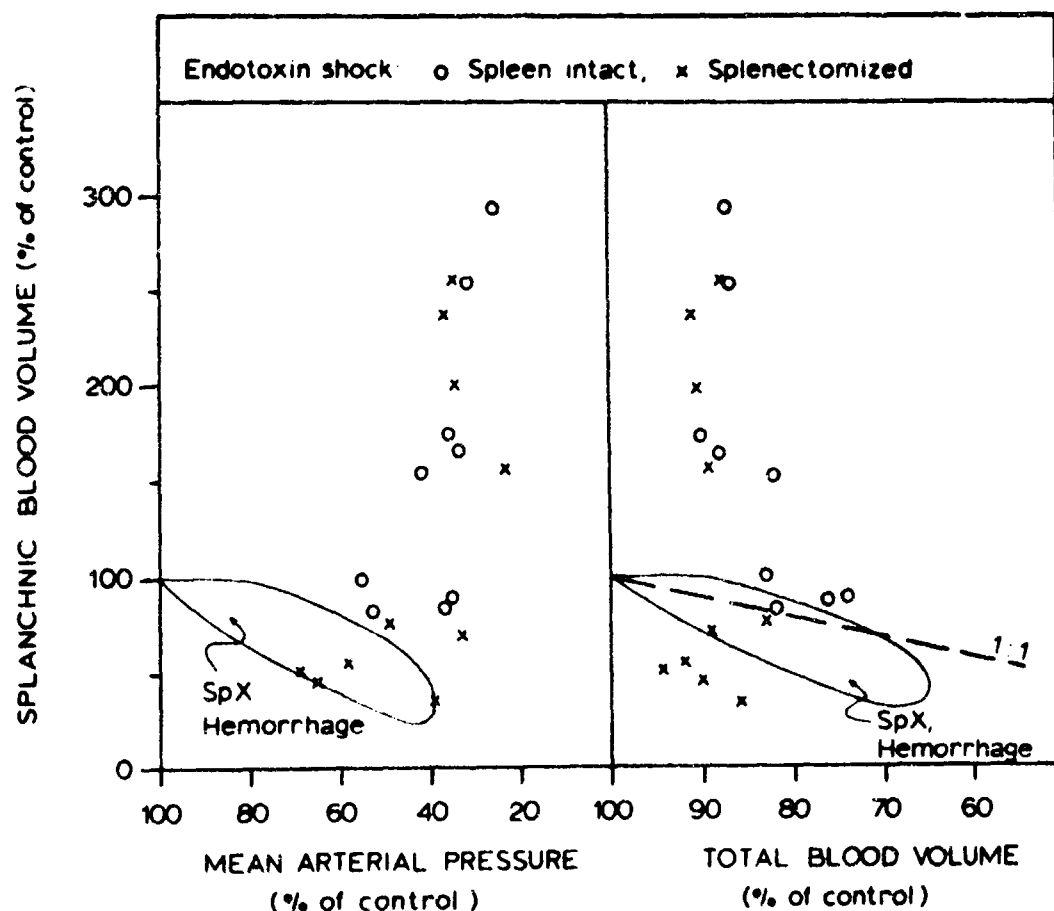


Fig. 11. Splanchnic blood volume in endotoxin shock, plotted against mean arterial pressure (left panel) and total blood volume (right panel). The results on splenectomized dogs after hemorrhage (areas enclosed by thin lines, unpublished data of Chien and Usami) are included for comparison. Note the progressive increase in splanchnic blood volume as the arterial pressure is reduced to less than 40-50% of control. The dotted line marked 1:1 in the right panel depicts the relationship expected if splanchnic blood volume were to decrease by the same proportion as the total blood volume. In endotoxin shock, especially for normal dogs (spleen intact), the splanchnic blood volume increases although the total blood volume is reduced.

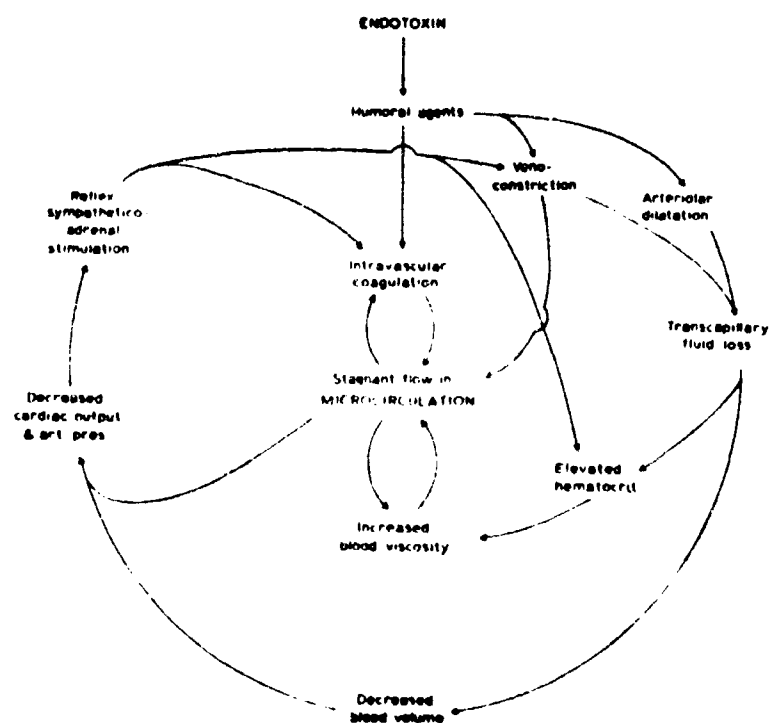


Fig. 12. A diagram showing the participation of various factors in constituting vicious cycles leading to circulatory deterioration in endotoxin shock.

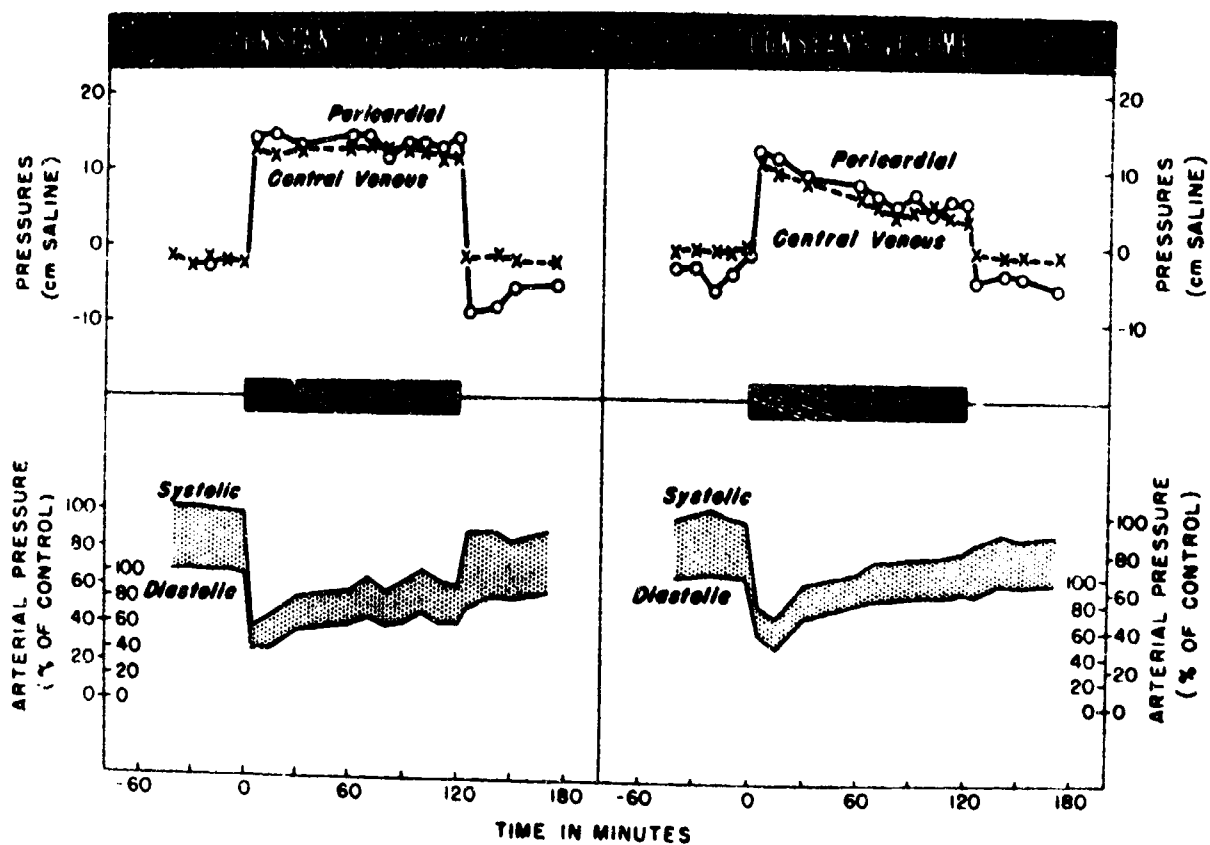


Fig. 13. Effect of constant pressure and constant volume pericardial tamponade on pericardial, central venous and arterial pressure.

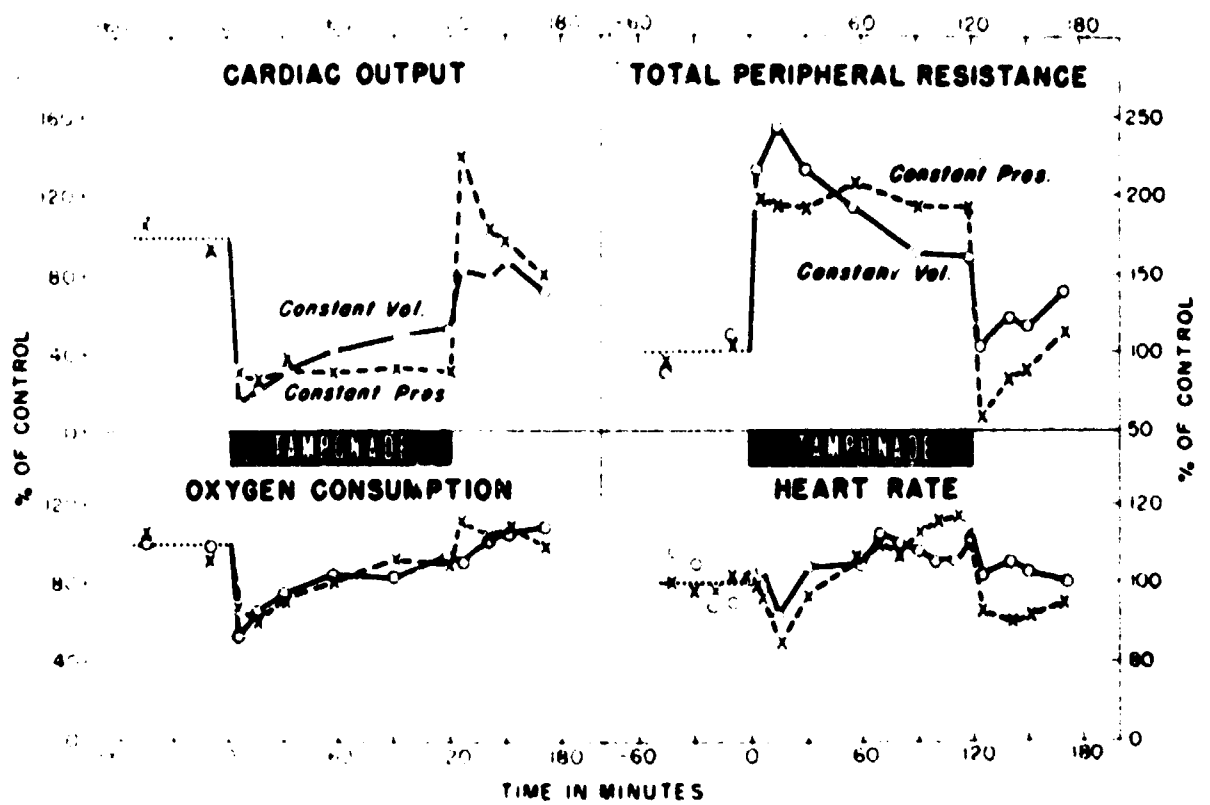


Fig. 14. Influence of constant pressure and constant volume pericardial tamponade on cardiac output, total peripheral resistance, oxygen consumption and heart rate.

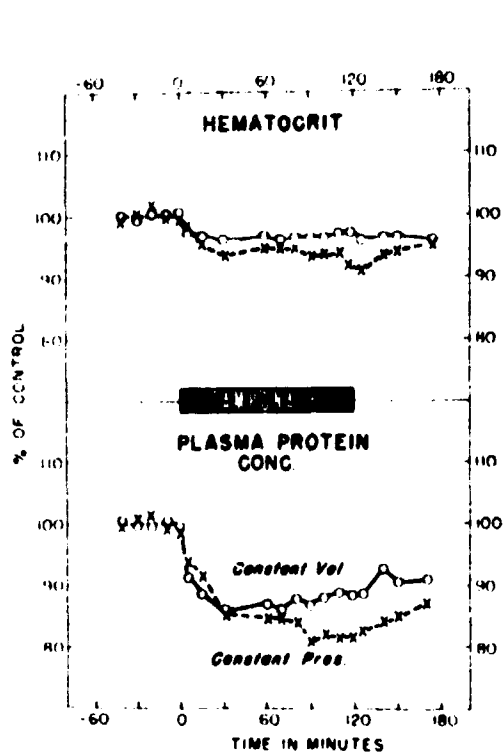


Fig. 15. Effect of constant pressure and constant volume pericardial tamponade on hematocrit and plasma protein concentration.

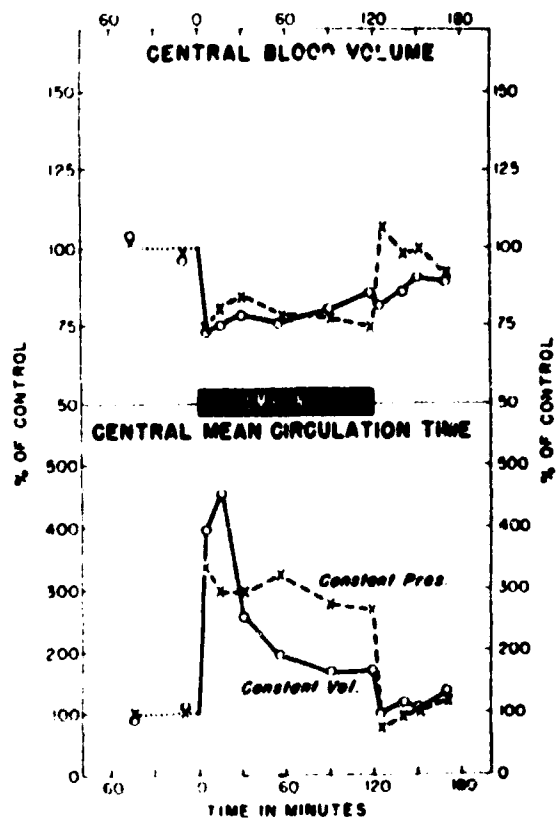


Fig. 16. Influence of constant pressure and constant volume pericardial tamponade on central blood volume and central mean circulation time.